REPUBLIQUE DU CAMEROUN
Paix – Travail – Patrie
****
UNIVERSITE DE YAOUNDE I
FACULTE DES SCIENCES
DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES
****
LABORATOIRE DE ZOOLOGIE

REPUBLIC OF CAMEROUN
Peace – Work – Fatherland
****
UNIVERSITY OF YAOUNDE I
FACULTY OF SCIENCE
DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY
****
LABORATORY OF ZOOLOGY

Seasonal polyphenism, temperature-induced plasticity and thermal stress adaptation in the tropical butterfly species Bicyclus dorothea (Cramer, 1779) across different regions of Cameroon

Thesis submitted in fulfilment of the requirement for the award of a Doctorate/Ph.D. degree in Biology of Animal Organisms

Par : **DONGMO KENFAK Michel Arnaud**
Master of Science

Sous la direction de
Timothy C. BONEBRAKE
Associate Professor
University of Hong Kong
Rachid HANNA
Principal Scientist
IITA-Cameroon

Année Académique : 2020

ATTTESTATION DE CORRECTION

UNIVERSITE DE YAOUNDE I
UNIVERSITY OF YAOUNDE I

FACULTE DES SCIENCES
FACULTY OF SCIENCE

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES
DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY

ATTESTATION DE CORRECTION

Nous soussignés, membres du jury de soutenance de la **Thèse de Doctorat/Ph.D** en Biologie des Organismes Animaux (Option Zoologie) de monsieur **DONGMO KENFAK Michel Arnaud,** matricule 09Q1460, soutenance autorisée par la correspondance N° 20912/UYI/VREPDTIC/DAAC/DEPE/SPD du Recteur de l'Université de Yaoundé I en date du 10 Juin 2020, attestons que les corrections exigées au candidat lors de cette évaluation faite le 25 Juin 2020, ont réellement été effectuées et que le présent document peut être déposé sous sa forme actuelle.

En foi de quoi cette attestation lui est délivrée pour servir et valoir de ce que de droit.

Yaoundé, le 23 JUL 2020

Président du Jury

Charles Félix Bilong Bilong
Professeur

Examinateur

Chef de Département

Charles Félix Bilong Bilong
Professeur

THE UNIVERSITY OF YAOUNDÉ I **Faculty of Science** Division of Programming and Follow-up of Academic Affairs		UNIVERSITE DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques
LIST OF PERMANENT TEACHING STAFF		**LISTE DES ENSEIGNANTS PERMANENTS**

ACADEMIC YEAR 2019/2020

(By Department and by Grade)

DATE UPDATED: 19 February 2020

ADMINISTRATION

DEAN: TCHOUANKEU Jean-Claude, *Associate Professor*

VICE-DEAN / DPSAA : DONGO Etienne, *Professor*

VICE-DEAN / DSSE : AJEAGAH Gideon AGHAINDUM, *Associate Professor*

VICE-DEAN / DRC : ABOSSOLO Monique, *Associate Professor*

Head of Administration and Financial Division (DAF): NDOYE FOE Marie C. F., *Associate Professor*

Head of Division of Education, Research and Academic Affairs (DAASR): MBAZE MEVA'A Luc Léonard, *Professor*

1- DEPARTMENT OF BIOCHEMISTRY (BCH) (38)			
N°	SURNAME AND GIVEN NAMES	GRADE	OBSERVATIONS
1	BIGOGA DIAGA Jude	Professor	In sevice
2	FEKAM BOYOM Fabrice	Professor	In service
3	FOKOU Elie	Professor	In service
4	KANSCI Germain	Professor	*Head of Department*
5	MBACHAM FON Wilfried	Professor	In service
6	MOUNDIPA FEWOU Paul	Professor	In service
7	NINTCHOM PENLAP V. épouse BENG	Professor	In service
8	OBEN Julius ENYONG	Professor	In service
9	ACHU Merci BIH	Associate Professor	In service
10	ATOGHO Barbara	Associate Professor	In service
11	AZANTSA KINGUE GABIN BORIS	Associate Professor	In service

12	BELINGA née NDOYE FOE M. C. F.	Associate Professor	*Head DAF / FS*
13	BOUDJEKO Thaddée	Associate Professor	In service
14	DJUIDJE NGOUNOUE Marcelline	Associate Professor	In service
15	EFFA NNOMO Pierre	Associate Professor	In service
16	NANA Louise épouse WAKAM	Associate Professor	In service
17	NGONDI Judith Laure	Associate Professor	In service
18	NGUEFACK Julienne	Associate Professor	In service
19	NJAYOU Frédéric Nico	Associate Professor	In service
20	MOFOR née TEUGWA Clotilde	Associate Professor	*IP Service MINESUP*
21	TCHANA KOUATCHOUA Angèle	Associate Professor	In service

22	AKINDEH MBUH NJI	Lecturer	In service
23	BEBOY EDZENGUELE Sara Nathalie	Lecturer	In service
24	DAKOLE DABOY Charles	Lecturer	In service
25	DJUIKWO NKONGA Ruth Viviane	Lecturer	In service
26	DONGMO LEKAGNE Joseph Blaise	Lecturer	In service
27	EWANE Cécile Anne	Lecturer	In service
28	FONKOUA Martin	Lecturer	In service
29	BEBEE Fadimatou	Lecturer	In service
30	KOTUE KAPTUE Charles	Lecturer	In service
31	LUNGA Paul KEILAH	Lecturer	In service
32	MANANGA Marlyse Joséphine	Lecturer	In service
33	MBONG ANGIE M. Mary Anne	Lecturer	In service
34	PACHANGOU NSANGOU Sylvain	Lecturer	In service
35	Palmer MASUMBE NETONGO	Lecturer	In service

36	MBOUCHE FANMOE Marceline Joëlle	Assistant	In service
37	OWONA AYISSI Vincent Brice	Assistant	In service
38	WILFRIED ANGIE Abia	Assistant	In service

2- DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (BPA) (46)			
1	AJEAGAH Gideon AGHAINDUM	Professor	*Vice-Dean/DSSE*
2	BILONG BILONG Charles-Félix	Professor	*Head of Department*
3	DIMO Théophile	Professor	In service
4	DJIETO-LORDON Champlain	Professor	In service
5	ESSOMBA née NTSAMA MBALA	Professor	*Vice-Dean/FMSB/UYI*
6	FOMENA Abraham	Professor	In service

7	KAMTCHOUING Pierre	Professor	In service
8	NJAMEN Dieudonné	Professor	In service
9	NJIOKOU Flobert	Professor	In service
10	NOLA Moïse	Professor	In service
11	TAN Paul VERNYUY	Professor	In service
12	TCHUEM TCHUENTE Louis Albert	Professor	*IP Service, Progr. Coord. / MINSANTE*
13	ZEBAZE TOGOUET Serge Hubert	Professor	In service

14	BILANDA Danielle Claude	Associate Professor	In service
15	DJIOGUE Séfirin	Associate Professor	In service
16	DZEUFIET DJOMENI Paul Désiré	Associate Professor	In service
17	JATSA BOUKENG Hermine épouse MEGAPTCHE	Associate Professor	In service
18	KEKEUNOU Sévilor	Associate Professor	In service
19	MEGNEKOU Rosette	Associate Professor	In service
20	MONY Ruth épouse NTONE	Associate Professor	In service
21	NGUEGUIM TSOFACK Florence	Associate Professor	In service
22	TOMBI Jeannette	Associate Professor	In service

23	ALENE Désirée Chantal	Lecturer	In service
24	ATSAMO Albert Donatien	Lecturer	In service
25	BELLET EDIMO Oscar Roger	Lecturer	In service
26	DONFACK Mireille	Lecturer	In service
27	ETEME ENAMA Serge	Lecturer	In service
28	GOUNOUE KAMKUMO Raceline	Lecturer	In service
29	KANDEDA KAVAYE Antoine	Lecturer	In service
30	LEKEUFACK FOLEFACK Guy B.	Lecturer	In service
31	MAHOB Raymond Joseph	Lecturer	In service
32	MBENOUN MASSE Paul Serge	Lecturer	In service
33	MOUNGANG Luciane Marlyse	Lecturer	In service
34	MVEYO NDANKEU Yves Patrick	Lecturer	In service
35	NGOUATEU KENFACK Omer Bébé	Lecturer	In service
36	NGUEMBOK	Lecturer	In service
37	NJUA Clarisse Yafi	Lecturer	*Head Division UBa*
38	NOAH EWOTI Olive Vivien	Lecturer	In service
39	TADU Zephyrin	Lecturer	In service
40	TAMSA ARFAO Antoine	Lecturer	In service
41	YEDE	Lecturer	In service

42	BASSOCK BAYIHA Etienne Didier	Assistant	In service
43	ESSAMA MBIDA Désirée Sandrine	Assistant	In service

44	KOGA MANG Debora	Assistant	In service
45	LEME BANOCK Lucie	Assistant	In service
46	YOUNOUSSA LAME	Assistant	In service

3- DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (BPV) (32)

1	AMBANG Zachée	Professor	*Head Division/UYII*
2	BELL Joseph Martin	Professor	In service
3	DJOCGOUE Pierre François	Professor	In service
4	MOSSEBO Dominique Claude	Professor	In service
5	YOUMBI Emmanuel	Professor	*Head of Department*
6	ZAPFACK Louis	Professor	In service

7	ANGONI Hyacinthe	Associate Professor	In service
8	BIYE Elvire Hortense	Associate Professor	In service
9	KENGNE NOUMSI Ives Magloire	Associate Professor	In service
10	MALA Armand William	Associate Professor	In service
11	MBARGA BINDZI Marie Alain	Associate Professor	CT/Minesup
12	MBOLO Marie	Associate Professor	In service
13	NDONGO BEKOLO	Associate Professor	*CE / MINRESI*
14	NGONKEU MAGAPTCHE Eddy L.	Associate Professor	In service
15	TSOATA Esaïe	Associate Professor	In service
16	TONFACK Libert Brice	Associate Professor	In service

17	DJEUANI Astride Carole	Lecturer	In service
18	GOMANDJE Christelle	Lecturer	In service
19	MAFFO MAFFO Nicole Liliane	Lecturer	In service
20	MAHBOU SOMO TOUKAM. Gabriel	Lecturer	In service
21	NGALLE Hermine BILLE	Lecturer	In service
22	NGOUO Lucas Vincent	Lecturer	In service
23	NNANGA MEBENGA Ruth Laure	Lecturer	In service
24	NOUKEU KOUAKAM Armelle	Lecturer	In service
25	ONANA JEAN MICHEL	Lecturer	In service

26	GODSWILL NTSOMBAH NTSEFONG	Assistant	In service
27	KABELONG BANAHO Louis-Paul-Roger	Assistant	In service
28	KONO Léon Dieudonné	Assistant	In service
29	LIBALAH Moses BAKONCK	Assistant	In service
30	LIKENG-LI-NGUE Benoit C.	Assistant	In service
31	TAEDOUNG Evariste Hermann	Assistant	In service
32	TEMEGNE NONO Carine	Assistant	In service

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (35)

1	AGWARA ONDOH Moïse	Professor	*Vice-Rector Univ. Ba*
2	ELIMBI Antoine	Professor	In service
3	Florence UFI CHINJE épouse MELO	Professor	*Vice Chancellor Univ. Ndere*
4	GHOGOMU Paul MINGO	Professor	*Minister in Charge of Special Duties P.R.*
5	NANSEU Njiki Charles Péguy	Professor	In service
6	NDIFON Peter TEKE	Professor	*CT MINRESI/Head of Department*
7	NGOMO Horace MANGA	Professor	*Vice Chancellor / UB*
8	NDIKONTAR Maurice KOR	Professor	*Vice-Dean Univ. Ba*
9	NENWA Justin	Professor	In service
10	NGAMENI Emmanuel	Professor	*DEAN FS UDs*

11	BABALE née DJAM DOUDOU	Associate Professor	*In Charge of Special Duties P.R.*
12	DJOUFAC WOUMFO Emmanuel	Associate Professor	In service
13	KAMGANG YOUBI Georges	Associate Professor	In service
14	KEMMEGNE MBOUGUEM Jean C.	Associate Professor	In service
15	KONG SAKEO	Associate Professor	In service
16	NDI NSAMI Julius	Associate Professor	In service
17	NJIOMOU C. épouse DJANGANG	Associate Professor	In service
18	NJOYA Dayirou	Associate Professor	In service
19	YOUNANG Elie	Associate Professor	In service

20	ACAYANKA Elie	Lecturer	In service
21	BELIBI BELIBI Placide Désiré	Lecturer	*C.S / ENS Bertoua*
22	CHEUMANI YONA Arnaud M.	Lecturer	In service
23	EMADACK Alphonse	Lecturer	In service
24	KENNE DEDZO GUSTAVE	Lecturer	In service
25	KOUOTOU DAOUDA	Lecturer	In service
26	MAKON Thomas Beauregard	Lecturer	In service
27	MBEY Jean Aime	Lecturer	In service
28	NCHIMI NONO KATIA	Lecturer	In service
29	NEBA nee NDOSIRI Bridget NDOYE	Lecturer	*CT / MINFEM*
30	NYAMEN Linda Dyorisse	Lecturer	In service
31	PABOUDAM GBAMBIE A.	Lecturer	In service
32	TCHAKOUTE KOUAMO Hervé	Lecturer	In service

33	NJANKWA NJABONG N. Eric	Assistant	In service
34	PATOUOSSA ISSOFA	Assistant	In service
35	SIEWE Jean Mernoz	Assistant	In service

5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (35)

1	DONGO Etienne	Professor	*Vice-DEAN / PSAA*
2	GHOGOMU TIH Robert Ralph	Professor	*Dir. IBAF/UDA*
3	NGOUELA Silvère Augustin	Professor	*Head of Department UDs*
4	NKENGFACK Augustin Ephreïm	Professor	*Head of Department*
5	NYASSE Barthélemy	Professor	In service
6	PEGNYEMB Dieudonné Emmanuel	Professor	*Director/ MINESUP*
7	WANDJI Jean	Professor	In service

8	Alex de Théodore ATCHADE	Associate Professor	*DEPE/ Rectorat/UYI*
9	EYONG Kenneth OBEN	Associate Professor	*Head Service DPER*
10	FOLEFOC Gabriel NGOSONG	Associate Professor	In service
11	FOTSO WABO Ghislain	Associate Professor	In service
12	KEUMEDJIO Félix	Associate Professor	In service
13	KEUMOGNE Marguerite	Associate Professor	In service
14	KOUAM Jacques	Associate Professor	In service
15	MBAZOA née DJAMA Céline	Associate Professor	In service
16	MKOUNGA Pierre	Associate Professor	In service
17	NOTE LOUGBOT Olivier Placide	Associate Professor	*Head Service / MINESUP*
18	NGO MBING Joséphine	Associate Professor	*S/Direct. MINERESI*
19	NGONO BIKOBO Dominique Serge	Associate Professor	In service
20	NOUNGOUE TCHAMO Diderot	Associate Professor	In service
21	TABOPDA KUATE Turibio	Associate Professor	In service
22	TCHOUANKEU Jean-Claude	Associate Professor	*DEAN /FS/ UYI*
23	TIH née NGO BILONG E. Anastasie	Associate Professor	In service
24	YANKEP Emmanuel	Associate Professor	In service

25	AMBASSA Pantaléon	Lecturer	In service
26	KAMTO Eutrophe Le Doux	Lecturer	In service
27	MVOT AKAK Carine	Lecturer	In service
28	NGNINTEDO Dominique	Lecturer	In service
29	NGOMO Orléans	Lecturer	In service
30	OUAHOUO WACHE Blandine M.	Lecturer	In service
31	SIELENOU TEDJON Valérie	Lecturer	In service
32	TAGATSING FOTSING Maurice	Lecturer	In service
33	ZONDENDEGOUMBA Ernestine	Lecturer	In service

34	MESSI Angélique Nicolas	Assistant	In service
35	TSEMEUGNE Joseph	Assistant	In service

6- DEPARTMENT OF COMPUTER SCIENCE (IN) (26)			
1	ATSA ETOUNDI Roger	Professor	*Head Div. MINESUP*
2	FOUDA NDJODO Marcel Laurent	Professor	*Head Dpt. ENS/Head IGA.MINESUP*
3	NDOUNDAM Réné	Associate Professor	In service
4	AMINOU Halidou	Lecturer	In service
5	DJAM Xaviera YOUHEP KIMBI	Lecturer	In service
6	EBELE Serge	Lecturer	In service
7	KOUOKAM KOUOKAM E. A.	Lecturer	In service
8	MELATAGIA YONTA Paulin	Lecturer	In service
9	MOTO MPONG Serge Alain	Lecturer	In service
10	TAPAMO Hyppolite	Lecturer	In service
11	ABESSOLO ALO'O Gislain	Lecturer	In service
12	KAMGUEU Patrick Olivier	Lecturer	In service
13	MONTHE DJIADEU Valery M.	Lecturer	In service
14	OLLE OLLE Daniel Claude Delort	Lecturer	*Head Dpt. Enset Ebolowa*
15	TINDO Gilbert	Lecturer	In service
16	TSOPZE Norbert	Lecturer	In service
17	WAKU KOUAMOU Jules	Lecturer	In service
18	BAYEM Jacques Narcisse	Assistant	In service
19	DOMGA KOMGUEM Rodrigue	Assistant	In service
20	EKODECK Stéphane Gael Raymond	Assistant	In service
21	HAMZA Adamou	Assistant	In service
22	JIOMEKONG AZANZI Fidel	Assistant	In service
23	MAKEMBE. S. Oswald	Assistant	In service
24	MESSI NGUELE Thomas	Assistant	In service
25	MEYEMDOU Nadège Sylvianne	Assistant	In service
26	NKONDOCK. MI. BAHANACK.N.	Assistant	In service

7- DEPARTMENT OF MATHEMATICS (MA) (30)			
1	EMVUDU WONO Yves S.	Professor	*Head Dept. Comp. Sci/ Inspector MINESUP*
2	AYISSI Raoult Domingo	Associate Professor	*Head of Department*
3	NKUIMI JUGNIA Célestin	Associate Professor	In service
4	NOUNDJEU Pierre	Associate Professor	In service
5	MBEHOU Mohamed	Associate Professor	In service
6	TCHAPNDA NJABO Sophonie B.	Associate Professor	*Director / AIMS Rwanda*

7	AGHOUKENG JIOFACK Jean Gérard	Lecturer	*Head Service MINPLAMAT*
8	CHENDJOU Gilbert	Lecturer	In service
9	DJIADEU NGAHA Michel	Lecturer	In service
10	DOUANLA YONTA Herman	Lecturer	In service
11	FOMEKONG Christophe	Lecturer	In service
12	KIANPI Maurice	Lecturer	In service
13	KIKI Maxime Armand	Lecturer	In service
14	MBAKOP Guy Merlin	Lecturer	In service
15	MBANG Joseph	Lecturer	In service
16	MBELE BIDIMA Martin Ledoux	Lecturer	In service
17	MENGUE MENGUE David Joe	Lecturer	In service
18	NGUEFACK Bernard	Lecturer	In service
19	NIMPA PEFOUNKEU Romain	Lecturer	In service
20	POLA DOUNDOU Emmanuel	Lecturer	In service
21	TAKAM SOH Patrice	Lecturer	In service
22	TCHANGANG Roger Duclos	Lecturer	In service
23	TCHOUNDJA Edgar Landry	Lecturer	In service
24	TETSADJIO TCHILEPECK M. E.	Lecturer	In service
25	TIAYA TSAGUE N. Anne-Marie	Lecturer	In service

26	MBIAKOP Hilaire George	Assistant	In service
27	BITYE MVONDO Esther Claudine	Assistant	In service
28	MBATAKOU Salomon Joseph	Assistant	In service
29	MEFENZA NOUNTU Thiery	Assistant	In service
30	TCHEUTIA Daniel Duviol	Assistant	In service

8- DEPARTMENT OF MICROBIOLOGY (MIB) (18)

1	ESSIA NGANG Jean Justin	Professor	*DRV/IMPM*

2	BOYOMO ONANA	Associate Professor	In service
3	NWAGA Dieudonné M.	Associate Professor	In service
4	NYEGUE Maximilienne Ascension	Associate Professor	In service
5	RIWOM Sara Honorine	Associate Professor	In service
6	SADO KAMDEM Sylvain Leroy	Associate Professor	In service

7	ASSAM ASSAM Jean Paul	Lecturer	In service
8	BODA Maurice	Lecturer	In service
9	BOUGNOM Blaise Pascal	Lecturer	In service
10	ESSONO OBOUGOU Germain G.	Lecturer	In service
11	NJIKI BIKOÏ Jacky	Lecturer	In service
12	TCHIKOUA Roger	Lecturer	In service

13	ESSONO Damien Marie	Assistant	In service
14	LAMYE Glory MOH	Assistant	In service
15	MEYIN A EBONG Solange	Assistant	In service
16	NKOUDOU ZE Nardis	Assistant	In service
17	SAKE NGANE Carole Stéphanie	Assistant	In service
18	TOBOLBAI Richard	Assistant	In service

9. DEPARTEMENT OF PHYSICS (PHY) (40)

1	BEN- BOLIE Germain Hubert	Professor	In service
2	ESSIMBI ZOBO Bernard	Professor	In service
3	KOFANE Timoléon Crépin	Professor	In service
4	NANA ENGO Serge Guy	Professor	In service
5	NDJAKA Jean Marie Bienvenu	Professor	*Head of Department*
6	NOUAYOU Robert	Professor	In service
7	NJANDJOCK NOUCK Philippe	Professor	*S/Director/ MINRESI*
8	PEMHA Elkana	Professor	In service
9	TABOD Charles TABOD	Professor	*DEAN Univ/Bda*
10	TCHAWOUA Clément	Professor	In service
11	WOAFO Paul	Professor	In service

12	BIYA MOTTO Frédéric	Associate Professor	*DG/HYDRO Mekin*
13	BODO Bertrand	Associate Professor	In service
14	DJUIDJE KENMOE épouse ALOYEM	Associate Professor	In service
15	EKOBENA FOUDA Henri Paul	Associate Professor	*Chef Division. UN*
16	EYEBE FOUDA Jean sire	Associate Professor	In service
17	FEWO Serge Ibraïd	Associate Professor	In service
18	HONA Jacques	Associate Professor	In service
19	MBANE BIOUELE César	Associate Professor	In service
20	NANA NBENDJO Blaise	Associate Professor	In service
21	NDOP Joseph	Associate Professor	In service
22	SAIDOU	Associate Professor	*MINRESI*
23	SIEWE SIEWE Martin	Associate Professor	In service
24	SIMO Elie	Associate Professor	In service
25	VONDOU Derbetini Appolinaire	Associate Professor	In service
26	WAKATA née BEYA Annie	Associate Professor	*S/ Director/* MINESUP
27	ZEKENG Serge Sylvain	Associate Professor	In service

28	ABDOURAHIMI	Lecturer	In service
29	EDONGUE HERVAIS	Lecturer	In service
30	ENYEGUE A NYAM spouse BELINGA	Lecturer	In service
31	FOUEDJIO David	Lecturer	*Head Cell. MINADER*

32	MBINACK Clément	Lecturer	In service
33	MBONO SAMBA Yves Christian U.	Lecturer	In service
34	MELI'I Joelle Larissa	Lecturer	*In service*
35	MVOGO ALAIN	Lecturer	*In service*
36	OBOUNOU Marcel	Lecturer	*DA/ Inter- State Univ /Sangmalima*
37	WOULACHE Rosalie Laure	Lecturer	In service

38	AYISSI EYEBE Guy François Valérie	Assistant	In service
39	CHAMANI Roméo	Assistant	In service
40	TEYOU NGOUPOU Ariel	Assistant	In service

10- DEPARTMENT OF EARTH SCIENCE (ST) (43)

1	BITOM Dieudonné	Professor	*DEAN / FASA / UDs*
2	FOUATEU Rose épse YONGUE	Professor	In service
3	KAMGANG Pierre	Professor	In service
4	NDJIGUI Paul Désiré	Professor	*Head of Department*
5	NDAM NGOUPAYOU Jules-Remy	Professor	In service
6	NGOS III Simon	Professor	DAAC/Uma
7	NKOUMBOU Charles	Professor	In service
8	NZENTI Jean-Paul	Professor	In service

9	ABOSSOLO née ANGUE Monique	Associate Professor	*Vice-Dean / DRC*
10	GHOGOMU Richard TANWI	Associate Professor	*Head Dpt. /UMa*
11	MOUNDI Amidou	Associate Professor	*CT./ MINIMDT*
12	NGUEUTCHOUA Gabriel	Associate Professor	*CEA/MINRESI*
13	NJILAH Isaac KONFOR	Associate Professor	In service
14	ONANA Vincent Laurent	Associate Professor	In service
15	BISSO Dieudonné	Associate Professor	*Dir Memve'ele Dam*
16	EKOMANE Emile	Associate Professor	*In service*
17	GANNO Sylvestre	Associate Professor	In service
18	NYECK Bruno	Associate Professor	In service
19	TCHOUANKOUE Jean-Pierre	Associate Professor	In service
20	TEMDJIM Robert	Associate Professor	In service
21	YENE ATANGANA Joseph Q.	Associate Professor	*Head Div. /MINTP*
22	ZO'O ZAME Philémon	Associate Professor	*DG/ART*

23	ANABA ONANA Achille Basile	Lecturer	*In service*
24	BEKOA Etienne	Lecturer	*In service*
25	ELISE SABABA	Lecturer	In service
26	ESSONO Jean	Lecturer	*In service*

27	EYONG JOHN TAKEM	Lecturer	In service
28	FUH Calistus Gentry	Lecturer	*State Sec. /MINMIDT*
29	LAMILEN BILLA Daniel	Lecturer	In service
30	MBESSE CECILE OLIVE	Lecturer	In service
31	MBIDA YEM	Lecturer	*In service*
32	METANG Victor	Lecturer	In service
33	MINYEM Dieudonné-Lucien	Lecturer	*Head Dept./Uma*
34	NGO BELNOUN Rose Noël	Lecturer	In service
35	NGO BIDJECK Louise Marie	Lecturer	In service
36	NOMO NEGUE Emmanuel	Lecturer	In service
37	NTSAMA ATANGANA Jacqueline	Lecturer	In service
38	TCHAKOUNTE J. épouse NOUMBEM	Lecturer	*Head. cell / MINRESI*
39	TCHAPTCHET TCHATO De P.	Lecturer	In service
40	TEHNA Nathanaël	Lecturer	In service
41	TEMGA Jean Pierre	Lecturer	In service

42	FEUMBA Roger	Assistant	In service
43	MBANGA NYOBE Jules	Assistant	In service

Numerical breakdown of permanent teachers of the Faculty of Science of the University of Yaoundé I

NUMBER OF TEACHERS					
DEPARTMENT	**Professors**	**Associate Professors**	**Lecturers**	**Assistants**	**Total**
BCH	9 (01)	13 (09)	14 (05)	03 (02)	**36 (16)**
BPA	13 (01)	09 (06)	19 (05)	05 (02)	**46 (13)**
BPV	06 (0)	10 (02)	09 (04)	07 (01)	**31 (09)**
CI	10 (01)	09 (02)	13 (02)	02 (0)	**35 (05)**
CO	07 (0)	17 (04)	09 (03)	03 (0)	**35 (07)**
IN	02 (0)	01 (0)	14 (01)	10 (02)	**26 (03)**
MAT	01 (0)	05 (0)	19 (01)	05 (01)	**30 (02)**
MIB	01 (0)	05 (02)	06 (01)	06 (02)	**17 (05)**
PHY	11 (0)	16 (01)	10 (03)	03 (0)	**40 (04)**
ST	08 (01)	14 (01)	19 (04)	02 (0)	**43 (06)**
Total	**68 (4)**	**99 (27)**	**132 (29)**	**45 (10)**	**344 (70)**

Total 344 (70) with:
- Professors 68 (04)
- Associate Professors 99 (27)
- Lecturers 132 (39)
- Assistants 46 (10)

() = Number of Women

DEDICATION

I dedicate this thesis to my wife Nadia Karelle TOUKEM and my daughter Danielle DONGMO NGUEGANG for all their support, patience and love.

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LIST OF ABBREVIATIONS

IITA: International Institute of Tropical Agriculture

CBI: Congo Basin Institute

CARN: Conservation Action Research Network

CBGP: Congo Basin Grant Program

UCLA: University of California, Los Angeles

PCR: Polymerase Chain Reaction

SNP: Single Nucleotide Polymorphism

PIRE: Professional International Research Education

PTTH: Prothoracicotropic hormone

Ctmin: Critical Thermal Minimum

Ctmax: Critical Thermal Maximum

TR: Tolerance Range

HKDT: Heat Knock Down Time

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ABSTRACT

Many organisms exhibit changes in phenotypic traits as a response to seasonal environmental variation. In the tropics, this seasonal environmental variation is characterized by the alternative wet and dry seasons which is challenging to cold-blooded animals due to their wide distribution in various ecosystem types. Further, these environmental changes have been accentuated during the last three decades because of the accelerated global warming to which tropical species are predicted to be more vulnerable than species occurring in temperate zones. Ectotherms found in naturally low thermal variation habitats like tropical forests may be more vulnerable to climate change impacts than high climate fluctuating habitats, but the extent to which plasticity can buffer warming impacts is not well known. This study investigates few bio-ecological aspects of the light bush brown butterfly, *Bicyclus dorothea* (Cramer, 1779), the role of habitat in generating seasonal polyphenism in different populations of this butterfly and the pattern of plasticity when it is submitted to various development temperatures in the laboratory. Also, this thesis assessed the ability of different *B. dorothea* population to withstand stressful temperatures imposed under laboratory conditions.

A survey was conducted in thirty-two localities of Cameroon to assess the presence/absence data of the butterfly and to explore some aspects of its biology and ecology. The response of adult *B. dorothea* to seasonality in four localities under natural conditions was assessed by evaluating the variation of their wing morphology throughout seasons. To that end, butterflies were captured during the wet and dry seasons across four localities representing two distinct habitats, namely forest and ecotone (forest–savanna transition zone) over a 2-years period (2015–2016). Morphological measurements (such as the diameter of the wing spots) were then conducted using a stereomicroscope and a caliper on thirteen wing pattern elements of each individual captured in the field. Further, the study also associated a common garden approach by assessing the phenotypic variation in some life history traits and wing pattern elements of the second-generation individuals from all the populations reared at three constant temperature regimes (22, 26 and 30ºC). The thermal reaction norm method was used to evaluate if the phenotypic plasticity observed under field conditions was conserved when individuals were reared under laboratory conditions. The stress resistance was evaluated by assessing physiological traits such as the critical thermal minimum and maximum (Ctmin and Ctmax), the heat knock down and the recovery time after exposure to heat. A set of statistical tools such as the principal component analysis was used to quantify the variation of each wing pattern over the two years of sampling at each locality.

The survey revealed that *B. dorothea* is widely distributed in various law to mid-altitude forest types in Cameroon. In the wild, seasonal polyphenism in wing pattern elements was found in all the populations evaluated. However, populations from ecotone habitat showed strong phenotypic variation in the diameter of their wing spot in comparison to their forest counterparts. Individuals exhibit larger wing spot during the wet season and very reduced ones in the dry season. The temperature-induced plasticity has shown that some morphological and life history traits were conserved under the range of constant developing temperatures selected in the laboratory in all population tested. Regarding the thermal tolerance, second-generation individuals originating from the ecotone, reared under conditions common to both populations, exhibited higher upper thermal limits (Ctmax) than individuals originating from forest (about 3ºC greater); the same trend was observed for heat stress and recovery time. Lower thermal limits (Ctmin) were also lower for the ecotone populations but only slightly (about 1ºC).

The present research has demonstrated that the plastic response and thermal tolerance of different populations of *B. dorothea* are habitat-specific. Thermally variable forest-savanna mosaic populations of *B. dorothea* are more plastic in traits evaluated than stable tropical forest ones. These results can be extended to other cool blooded animals living in different tropical habitats and it is important because it will help mitigating and better understand the harmful effects of ongoing climate change on insects' populations in Central Africa.

Keywords: seasonal polyphenism, phenotypic plasticity, thermal stress adaptation, *Bicyclus dorothea*, forest, ecotone.

RESUME

Les variations environnementales observées au cours des saisons peuvent être à l'origine des changements de certains traits phénotypiques chez certains organismes. Sous les tropiques, ces variations environnementales sont caractérisées par une alternance des saisons sèches et pluvieuses pouvant induire des variations morphologiques ou physiologiques chez les ectothermes. Ces changements environnementaux se sont accentués au cours des trois dernières décennies en raison de l'accélération des phénomènes liés aux changements climatiques auxquels les espèces tropicales sont vulnérables. L'accommodation au stress thermique est structurée par les conditions environnementales expérimentées par les individus tout au long de leur ontogenèse. A ce titre, les ectothermes vivant dans les paysages à faible variation thermique, tels que les forêts tropicales, peuvent donc être plus vulnérables aux impacts du réchauffement climatique que les espèces des paysages soumis à de fortes fluctuations thermiques. Le présent travail examine quelques aspects de la bioécologie du papillon *Bicyclus dorothea* (Cramer, 1779), le rôle des types de paysages sur le polyphénisme saisonnier des différentes populations de ce papillon et la plasticité phénotypique induite par la température au laboratoire. Il est également question d'évaluer la capacité de différentes populations de *B. dorothea* à résister à des températures stressantes imposées dans les conditions de laboratoire.

Un suivi a été mené dans trente-deux localités du Cameroun pour connaître un peu plus la bio-écologie de *B. dorothea*. Les variations morphologiques saisonnières des adultes de *B. dorothea* ont été évaluées en mesurant certains paramètres alaires tels que le diamètre des ocelles et la longueur des ailes dans quatre localités (Ako, Ndikiniméki, Mbalmayo et Somalomo) sur une période de deux ans (2015-2016). En outre, cette étude a également porté sur la variation phénotypique de certains traits de vie et de la morphologie des ailes chez des individus de chaque population élevée sous trois régimes de températures constantes (22, 26 et 30°C). L'analyse de la norme de réaction a été utilisée pour vérifier si la plasticité phénotypique observée dans les conditions naturelles était conservée lorsque les individus étaient élevés dans des conditions de laboratoire. La résistance au stress thermique a été analysée en mesurant les caractéristiques physiologiques telles que les températures minimales et maximales critiques (Ctmin et Ctmax), « le heat knock down » et le temps de réveil après exposition des papillons adultes à la chaleur. Un ensemble d'outils statistiques tels que l'analyse en composantes principales, l'analyse de variances ont été utilisés pour quantifier les réponses plastiques de chaque population aux conditions environnementales.

Le suivi biologique a révélé que *B. dorothea* est largement répandu au Cameroun, dans divers types de forêts de basses et moyennes altitudes. Dans la nature, un polyphénisme saisonnier de la morphologie des ailes a été observé chez toutes les populations étudiées. Les populations des écotones ont montré une forte variation phénotypique du diamètre de leurs ocelles alaires par rapport aux populations des paysages forestiers. En effet, les individus présentent des ocelles plus larges pendant la saison des pluies alors qu'en saison sèche ces ocelles sont très réduits. La plasticité phénotypique induite par la température a montré que certains traits de vie ont été conservés dans la gamme de températures de développement au laboratoire chez toutes les populations testées. En ce qui concerne la tolérance thermique, les individus de la deuxième génération issus des écotones présentent des limites thermiques supérieures (Ctmax) plus élevées (environ 3°C de plus) que les individus provenant de forêts; la même tendance a été observée pour le « heat knock down » et le temps de réveil après le stress thermique. Les limites thermiques inférieures (Ctmin) ont également été plus faibles pour les populations des écotones (environ 1ºC de plus) mais ne présentaient pas une variabilité statistiquement significative.

La présente étude a démontré que la réponse plastique et la tolérance thermique de différentes populations de *B. dorothea* sont spécifiques à chaque population ainsi qu'au type de paysage. Les populations de *B. dorothea* des paysages au climat très fluctuant sont plus plastiques que celles de forêts au climat plus stable. Ces résultats peuvent être étendus à d'autres ectothermes vivant dans les zones tropicales et sont importants, car ils aident à mieux comprendre les effets néfastes des changements climatiques en cours sur les populations d'insectes en Afrique centrale.

Mots-clés : polyphénisme saisonnier, plasticité phénotypique, adaptation au stress thermique, *Bicyclus dorothea*, forêt, écotone.

INTRODUCTION

The concept of geographic variation in phenotypes has a long history in evolutionary biology, eventually leading Darwin and Wallace to simultaneously conceive the theory of evolution by natural selection (Darwin and Wallace, 1858; Darwin, 1859). Living organisms inhabit environments that vary in time and space, and therefore undergo changing selection pressure. Almost all environments are generally characterized by cyclic climatic events representing seasons which are substantially different when considering some climate components. In tropical environments, these climate variabilities are represented by a variation in rainfall and/or temperature exerting a major influence on the alternating wet and dry seasons (Huntley and Walker, 2012). This environmental variability challenges the adaptation capacity of ectotherms in general and insects in particular. One of the central questions in ecology is how ectotherms adapt to changing environmental conditions. Insects generally respond to these variations through behavioral and/or physiological adaptations such as, migration or diapause and production of alternative phenotypes also known as phenotypic plasticity. Variation at the phenotypic level is commonly observed in morphological, physiological, life history and behavioral traits among populations of the same species from different geographical areas. Phenotypic plasticity can promote adaptive changes in morphology (Nylin and Gotthard, 1998) and/or variation in development time or growth rate (Gotthard *et al*., 1995). Environmental changes related to seasonality can profoundly impact insect life cycles and trigger morphological adaptations that generate seasonal polyphenism in which different phenotype predominate at different time of the season (Shapiro, 1976; Nijhout, 2003a).

Polyphenism is a phenomenon characterized by the production of two or more distinct phenotypes from a single genotype (Shapiro, 1976). It is thought to be an adaptive response and potentially a determining factor in the evolutionary success of insects (Simpson *et al*., 2011). This phenomenon is common in many insect species who exhibit this feature as a response to variation in a set of environmental cues (Simpson *et al*., 2011). In a seasonal environment, polyphenism is the result of contrasting but predictable cues, leading to different morphologies, life history traits or behaviors expressed in each season which allows species to persist in a given environment (David *et al*., 1997; Oostra *et al*., 2011). These environmental cues can be temporal, spatial, biotic or abiotic (Whitman and Agrawal, 2009).

In recent decades, multiple aspects of polyphenism in insects have been studied (Capy, 1993; David *et al*., 1997; Ribeiro and Freitas, 2011). Thus, attention has been paid to a range of Lepidoptera species such as *Melanitis leda* (Linneaus, 1758, see Brakefield and Larsen, 1984; Brakefield, 1987),

Manduca sexta (Linneaus, 1763, see Kingsolver *et al*., 2009), and *Automeris io* (Fabricius, 1775, see Sourakov, 2014). However, species belonging to the subtribe Mycalesina and the genus *Bicyclus* (Kirby, 1871) represent the best-studied taxa with respect to phenotypic plasticity in the field (Brakefield and Reitsma 1991, Windig *et al*., 1994, Brakefield and Frankino 2009, Brakefield and Zwaan, 2011). These species represent the best-studied taxa with respect to phenotypic plasticity in the field (Brakefield and Reitsma, 1991; Windig *et al*., 1994, Brakefield and Frankino, 2009; Brakefield and Zwaan, 2011) and under laboratory conditions (Nokelainen *et al*., 2017; van Bergen *et al*., 2017). Studies in phenotypic plasticity have used many *Bicyclus* species such as *Bicyclus anynana* (De Jong *et al*., 2010), *Bicyclus cottrelli*, *Bicyclus safitza*, *Bicyclus ena*, *Bicyclus vulgaris* and *Bicyclus vansoni* (Windig *et al*., 1994; Roskam and Brakefield, 1996), and *Bicyclus sanaos* (Oostra *et al*., 2014; van Bergen *et al*., 2017). Studies conducted for example in East and Southern Africa have shown that during the dry season (characterized by scarce nutritive resources and a drop of monthly mean temperatures) adult *B. anynana* typically express wings with small cryptic eyespots (Brakefield and Reistma, 1991; Brakefield, 1997). Moreover, apart from wing patterns, many other life history traits are also affected such as reproductive diapause, fat content, longevity, body weight, development time of immature stages, and predator avoidance (Kooi *et al*., 1997; Zwaan *et al*., 2001; Zijlstra *et al*., 2002; Lyytinen *et al*., 2004; Westerman and Monteiro, 2016). During the wet season, resources are abundant and adult butterflies display wings with prominent and concentric eyespots along the wings' distal margin. These favorable conditions allow butterflies to develop faster and produce two or three generations before the onset of the dry season. This polyphenism in *B. anynana* wing patterns appears to be mainly induced by temperature variation over wet (high temperature) and dry (low temperature) seasons in their natural habitat in Malawi (Brakefield *et al*., 2007).

Another important aspect in the ecology of polyphenic species is that they are distributed in a wide range of habitats which do not have necessarily the same climate pattern. In such conditions, genetically similar populations exposed to different seasonal environments may not have the same response to an environmental stimulus. Among these climatic components, temperature has been reported to be one of the most important factors in inducing plastic response in butterflies in general and Satyrines in particular (Brakefield and Reitsma, 1991; Windig, 1991a and 1991b). However, the genotype–environment interaction of different populations might affect plastic response among insects (Brakefield and Kesbeke, 1997). A practical approach to assess the effect of an environmental factor on some specific or multiple genotypes is to assess their plastic response under laboratory conditions (Atkinson, 1994; Geister *et al*., 2008). Such investigations have been carried out on

Satyrine butterflies to explore whether the genotype-environment interaction occurs for morphological and life history traits across constant temperature regime (Braby and Jones, 1994; Roskam and Brakefield, 1996; De Jong *et al*., 2010; Oostra *et al*., 2014; Brakefield, *et al*., 2014, Nokelainen *et al*., 2017; van Bergen *et al*., 2017).

In addition to seasonal polyphenism induced by environmental cues, ongoing climate warming is having important ecological consequences on biodiversity including local extinctions, population shifts, changes in community structure and composition, and changes in phenology (Easterling *et al*., 2000; Thomas *et al*., 2004; Parmesan, 2006; Scheffers *et al*., 2014). Recent physiological-based models have shown that tropical species, and tropical ectotherms, may be vulnerable to climate warming (Deutsch *et al*., 2008; Bonebrake and Deutsch, 2012; Sunday *et al*., 2012; Sinclair *et al*., 2016). However, existing studies have shown that the sensitivity of ectotherms to climate change is structured and is variable by their adaptation to experienced thermal variation and varies across altitude, latitude and habitat's characteristics (Gibert and Huey, 2001; Addai and Baidoo, 2013; Sunday *et al*. 2014; García-Robledo *et al*., 2016).

Habitat plays a key role in regulating thermal sensitivity in terrestrial ectotherms (Nowakowski *et al*., 2017; Landry-Yuan *et al*., 2018). Many of these ectotherms with a broad geographical range have their populations occurring in highly diverse habitat types. In such conditions, the selective pressure (for example climate or vegetation structure) might act differently and have significant repercussions on some of their physiological traits (Hoffmann *et al*., 2003). Local adaptation of ectotherms and their ability to withstand environmental changes are therefore habitat-dependent (Somero, 2010; Sanborn *et al*., 2011; Kaspari *et al*., 2015; Nadeau *et al*., 2017). Ectotherms inhabiting variable environments may be more tolerant to climate variation than populations or species living in more stable habitats (Frishkoff *et al*., 2015; Bonebrake *et al*., 2016). In fact, local temperature can be mediated by vegetation density and land cover (Cosentino *et al*., 2011). An increase of temperature at a regional level can be perceived differently by ectotherms dwelling in different vegetation or land cover types. Hence, within different habitat types, ectotherms can buffer extreme temperatures using different levels of thermal refugia or shelter (Walther *et al*., 2002; Parmesan, 2006; Scheffers *et al*., 2014). Different types of responses of species to recent global warming have been explored and can involve shift in species' ranges, phenology modification, community structure and phenotypic plasticity (Walther *et al*., 2002; Parmesan, 2006).

Since animal populations in natural environment generally vary in space and time, they undergo different environmental pressures and phenotypic plasticity is usually used by these

populations to face harsh conditions (Whitman and Ananthakrishnan, 2009; Merilä and Hendry, 2014; Valladares *et al*., 2014). However, the time needed by the environmental cue and the plastic response are important to confirm the reliability of cues for predicting future environmental conditions and the fitness consequences of plasticity because some forms of developmental plasticity can alter its adaptation value (Levins, 1968). Among plastic traits in ectotherms, physiological acclimation to temperature is well known and may occur under different contexts.

Among all the studies cited above as illustration of the plastic response of insects to seasonality or thermal stress adaptation to extreme weather, none of them have investigated the role of the habitat in generating phenotypic plasticity. When considering the variability of its climate and topography, Cameroon is one of the countries whose ecosystem diversity is no longer to be demonstrated. The main known biome in tropical Africa to which Cameroon belongs is rainforest, though it is sometime dotted with savannah patches and human-generated habitats as a result of deforestation. Beside this variation, forest generally meets with other ecological communities, ecosystems or ecological regions at their boundary forming a sort of community mosaic termed as ecotone (McArthur and Sanderson, 1999; Kark and Van Rensburg, 2006). Ecotones occur at multiple spatial scales and range from natural boundaries to human-generated ecotones. Recent studies suggested that species richness and abundance tend to peak at ecotone level, though exceptions may occur. Moreover, ecotones have also been stated as high speciation centers and hence many researchers have made it as their conservation priorities (Smith *et al*., 1997; Walker *et al*., 2003; Kark, 2007; Senft, 2009). One of the main contributions leading to the role habitat can play in generating divergent phenotypes was the research by Smith *et al*. (2004) on geographical variation in African rainforest passerine bird. Variation at the phenotypic level is commonly observed among populations of the same species from different geographical areas, in morphological, physiological, and behavioral traits. The forest-savannah ecotones of Cameroon offer the opportunity to address questions on their influence on seasonal polyphenism in wing pattern variation and thermal tolerance of insects in comparison with forest habitats.

The main objective of this study is to investigate the role of different habitats in structuring seasonal polyphenism, temperature-induced plasticity and thermal stress response in different populations of the light bush brown butterfly *Bicyclus dorothea* Cramer, 1779 in Cameroon. We examined the following key aspects:

- few bioecological aspects of *B. dorothea*;

- morphological variations in wing pattern among wild populations of *B. dorothea* from forests and forest-savannah mosaic;
- plastic response of some life history traits and wing pattern variations in *B. dorothea* from different habitat when reared at different temperature ranges;
- thermal stress adaptation in different populations of *B. dorothea*.

The work presented in this thesis combines studies on seasonal polyphenism, temperature induced plasticity and thermal stress adaptation in relation with *B. dorothea*. At the start of this work, an introduction gives the context and state the rationale of this study. In the first major part, a literature review gives all information on the actual knowledge on *B. dorothea*, phenotypic plasticity, reaction norms and different aspects of thermal stress adaptation in insects. The second part details the methods and the material used to achieve this study, followed by a section on results and discussion. This thesis concludes with highlights of salient results and recommendations for biological conservation under warming scenarios.

CHAPTER I:

LITERATURE REVIEW

I-1. The light bush brown butterfly, *Bicyclus dorothea* (Cramer, 1779)

I-1.1. Position of *Bicyclus dorothea* into the taxonomy of Lepidoptera

The light bush brown butterfly belongs to the class Hexapoda, order Lepidoptera, super-family Papilionoidea, family Nymphalidae, sub-family Satyrinae, tribe Elymniini, sub-tribe Mycalesine, genus *Bicyclus* and species *Bicyclus dorothea* (Cramer, 1779). The information related to the taxonomy of *B. dorothea* was obtained from the following literature: Larsen (2005), Vande Weghe (2010) and Aduse-Poku *et al*. (2015 and 2017).

General features of the class Hexapoda

- the body is segmented into three parts: the head, the thorax and the abdomen;
- the thorax bears three pairs of legs;

General features of the order Lepidoptera

Lepidopteran species are characterized by the following features:

- the body is covered by scales; the scales are modified flattened hairs, giving butterflies and moths their extraordinary variety of color and pattern;
- while mandibles or jaws (chewing mouthpart) are only present in larval stages, mouthparts of most adults Lepidoptera are the sucking type with a proboscis;
- almost all lepidopteran species have two pairs of membranous wings;

General features of the superfamily Papilionoidea

- the body is smaller and less moth-like;
- the antennae are straight and clubbed;
- the caterpillars do not spin cocoons in which they pupate.

General features of family Nymphalidae

- the forelegs of the adults are small and hairy, resembling tiny brushes, and are not used for walking which is why they are also known as brush-footed butterflies;
- the pattern of wing veins of the forewings is unique, and the rigid antennae are tripped with little knobs called clubs;

- eggs vary in shape and in their arrangement on the plant. Caterpillars vary considerably in their appearance but are often hairy or spiny. Pupae have a cremaster from which they are suspended upside down but have no silk girdle and form no cocoon.

General features of subfamily Satyrinae

- caterpillars feed mostly on monocotyledonous plants such as elephant grass (Genus *Pennisetum*), lawn (*Axonopus*, *Paspalum*);
- they have larvae with bifid tails; adults frequently have large eyespots on the ventral and sometimes dorsal surfaces of the wings;

General features of the genus *Bicyclus* Kirby, 1871

- presence of hairs on the eyes;
- the upper sides of the wings are brown, often with one or more eyespots on the forewings;
- all males have androconial organs of various visible types.

General features of *Bicyclus dorothea* (Cramer, 1779)

Bicyclus dorothea has the following characters:

- it is one of the lightest of the genus although it is very similar to other members of the "*dorothea*-complex" such as *Bicyclus moyses* Condsmin & Fox, 1964 and *Bicyclus jefferyi* Fox, 1963. In males, the discal area of the forewings and the basal/costal half of the hindwing is lighter than the rest of the wing while in females, wings are uniformly grey;
- the male's hind wing bears two androconial brushes which are absent in females; this is one of the main features that can be used to differentiate *B. dorothea*, *B. moyses* and *B. jefferyi*. In fact, males of all three species have two androconial brushes in cell Rs and CuA2 of the hind wings, but in *B. dorothea* the brush is light brown to yellow and weakly developed in cell Rs while in *B. moyses* the brush is darker, and it is almost black in *B. jefferyi*;

I-1.2. Synonyms of *Bicyclus dorothea*

The classification of *B. dorothea* has evolved considerably since its first description. The species was described for the first time as *Mycalesis raesaces* Hewitson, 1866. The name changed overtime from *Papillio dorothea* Cramer, 1779, *Papillio melusina* Fabricius, 1787, *Papillio miriam*

Fabricius, 1793 and *Mycalesis raesaces* Hewitson, 1866. It was then later re-described as *Bicyclus dorothea* (Cramer, 1779) see Condamin (1973).

I-2. Bio-ecology of *Bicyclus dorothea*

I-2.1. Geographic distribution

Bicyclus butterflies are endemic to Africa; *Bicyclus dorothea* is found in the whole west and central African forest zone. Its distribution extends to southern Cameroon, the northern part of the Republic of Congo, Democratic Republic of Congo and Central African Republic (Aduse-Poku *et al*., 2017). Larsen (2005) and vande Weghe (2010) stated its presence in northern Angola, but a recent study states it is rather *B. moyses* which is present there (Aduse-Poku *et al*., 2017). In addition, none of these studies noted its presence in Gabon, but it has been recently recorded at the Minkébé National Park, northern Gabon (vande Weghe, pers. comm). The distribution of *B. dorothea* in Africa is given by Figure 1.

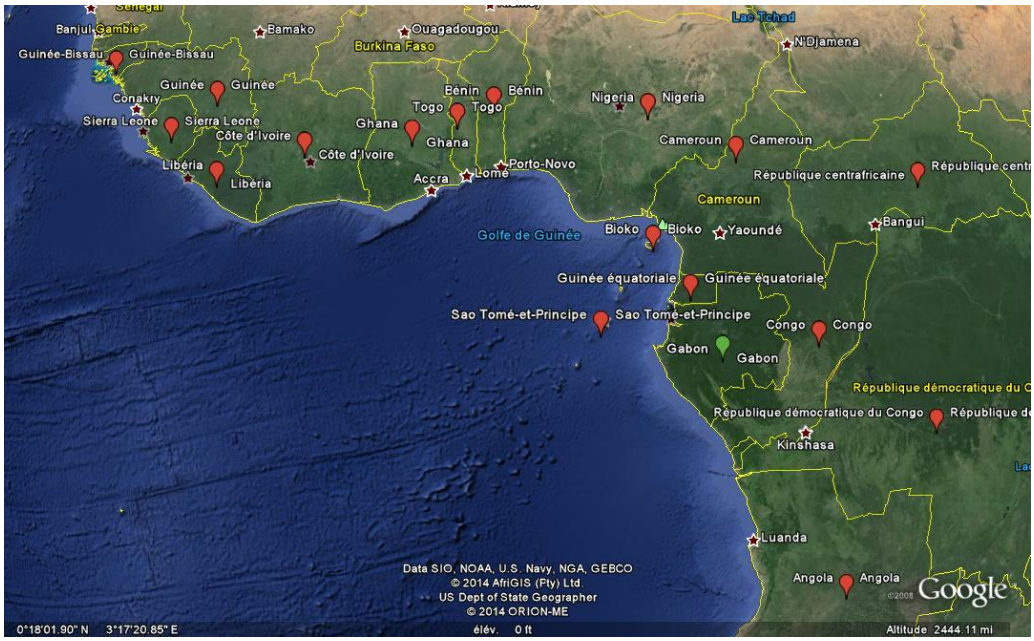

Figure 1: Distribution of *Bicyclus dorothea* in Africa (Larsen, 2005; vande Veghe, 2010).

I-2.2. Larval host plants and adult feeding

Larvae of the genus *Bicyclus* feed primarily on grasses (Poaceae). An artificial diet made up of bean flour has been developed and is successfully used to rear immature stages of *B. anynana*, *B. ena* and *B. safitza* (Holloway *et al*., 1991). Many species in the family Poaceae are used as host plants

by *B. dorothea*. Larvae have been reared on plants of the genera *Oplismenus*, *Paspalum* and *Axonopus* at Lamto, Cote d'Ivoire (Vuattoux, 1994) but all trials with maize (*Zea mays*) were unsuccessful in contrast to *B. anynana* for which maize is generally used as larval host plant in many research topics (Kooi, 1992).

Whilst most adult butterflies obtain carbohydrates by feeding on nectar (Bonebrake *et al*., 2010), satyrine species usually feed on decaying fruits (Boggs, 1997a and b). *Bicyclus dorothea* feeds on fallen fruits found in the forest such as guava, overripe banana, umbrella fruit, mangoes and many other species. Sometimes, individuals can exhibit mud-puddling behaviour, but they have never been observed foraging on dung or on nectar (Larsen, 2005).

I-2.3. Mating, reproduction and adult life span

Mating, reproduction, and adult life span have been well studied in a related satyrine species, *B. anynana* (Pijpe *et al*., 2007; Geister, 2008). These studies have highlighted the role of experimental set-up (Brakefield *et al*., 2009), dorsal eyespot and wing sizes (Breuker and Brakefield, 2002), sex pheromone production, sexual conflicts in males (Wiklund *et al*., 2001), sexual selection and the problem of protandry in reproduction (Zwaan *et al*., 2008; San Martin *et al*., 2011). In *B. anynana*, males and females are generally pooled together following eclosion in hanging cages for mating purposes. Females tend to be ready to mate after two or three days, after which they lay eggs during the two following weeks (Brakefield *et al*., 2009). Reproduction in *B. dorothea* appears to be like that observed with *B. anynana*, although some differences are apparent; for example, *B. dorothea* females generally do not mate for approximately 10 days after eclosion. Moreover, mating seems to be more challenging in *B. dorothea* than in *B. anynana* and sometimes it is difficult to produce viable eggs from lab-mated females. Almost all other species tested to date have a relatively long pre-oviposition period.

I-3. Phenotypic plasticity

I-3.1. Brief history and definition

In 1909, Johannsen was the first to distinguish conceptually and experimentally the two factors that contribute to the expression of an individual's characters: the genetic constitution to which he gave the name of "genotype" and the environmental factors that contribute with the first to achieve the "phenotype". During the same year, Woltereck (1909), studying morphological variations of parthenogenetic lineages of daphnia in relation to environmental factors (quantity of nutrients and

temperature), coined the term “reaction norm” shaping for the first time the concept of phenotypic plasticity even though it was not formal. Although the concept has been known for more than a century, it is the botanist Bradshaw (1965) who explicitly used the term phenotypic plasticity.

Numerous authors have defined phenotypic plasticity and these definitions seem similar. Bradshaw (1965) defined it as «the amount by which the expressions of individual characteristics of a genotype are changed by different environments». West-Eberhard (2003) thinks it is «a condition-sensitive development or the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity». Pigliucci (2005) termed it as «the property of a given genotype to produce different phenotypes in response to distinct environmental conditions». From these definitions, it can be seen that whatever the definition, phenotypic plasticity involves a genotype subjected to a changing environment. Phenotypic plasticity can be continuous, in which case it is called a reaction norm, or discontinuous, in which case it is called a polyphenism.

I-3.2. Traits that can exhibit phenotypic plasticity

Though the concept was first applied to morphological traits (Woltereck, 1909) and many authors are still linking it to morphology, phenotypic plasticity can be also applied to many traits among organisms. Thus, many other traits related to biochemistry, physiology, behavior, reproduction and life history can undergo changes in response to environmental variation and can then be analyzed in the angle of phenotypic plasticity (Whitman and Ananthakrishnan, 2009). Hence, phenomena such as heat shock proteins, acclimation, diapause, immunology, host-plant switching, enzyme induction, predator induced defense, maternal effects, homeostasis and mate choice can undergo phenotypic plasticity if the environment changes. Organisms are however known for the complexity and the integration of their systems. The variability of environment will not then induce the modification of only one trait but a suit of modifications that ranges among many independent and interconnected traits in the organism. An example is locust polyphenism, in which solitary and gregarious phenotypes differ in behavior, morphology, food selection, body color, gene expression, neuro-endocrine, and nutritional physiology, metabolism, immune responses, pheromone production, reproduction, and longevity (Simpson *et al*., 2005).

I-3.3. Conditions that favor phenotypic plasticity

Among conditions that favor phenotypic plasticity is environmental heterogeneity, predictability in environmental cues, and migration or dispersion (Pigliucci, 2001).

I-3.3.1. Environmental heterogeneity

It is usually stated in the literature that genetic variability and phenotypic plasticity are the two alternatives solutions used by ectotherms to face environmental heterogeneity (Pigliucci, 2005; Suzuki and Nijhout, 2006; Simpson *et al*., 2011). It seems clear that in a perfectly uniform environment, a fixed phenotype will be favored even in the absence of any cost or limit to phenotypic plasticity. One of the major factors favoring the evolution of phenotypic plasticity is therefore environmental variability. Environments can vary from a little to a larger scale, leading to different level of plasticity in organisms. Bradshaw and Hardwick (1989) attempted to study in plants the environmental conditions that would lead to the evolution of specialized ecotypes or flexible genotypes. They suggested that specialized ecotypes are expected when environmental conditions change and then remain relatively stable, whereas in case of frequent stress alternating with normal conditions one would rather expect the evolution of plastic genotypes. In general, theoretical studies based on optimization models agree that phenotypic plasticity should be favored in case of temporal variability of the environment and small-scale spatial variability.

I-3.3.2. Predictability of environmental cues

In addition to the heterogeneity of the environment, another important factor in the evolution of phenotypic plasticity is its predictability of environmental cues (Whitman and Agrawal, 2009). The unpredictability of environmental variability (especially of temporal type) disadvantages phenotypic plasticity. However, a stochastic environment (that is completely unpredictable) is not necessarily incompatible with phenotypic plasticity, which can then take the form of adaptive coin-flipping. The adequacy between the phenotype expressed and the environment encountered must also depend on the response time of the trait which must be as short as possible and therefore the advantage of plasticity will depend on the type of trait considered.

I-3.3.3. Migration or dispersion

Very few models have examined the effects of population structure on the evolution of phenotypic plasticity; temporal variability is generally modeled as acting at the level of a single population and populations are assumed to be panmictic. Some studies however, have investigated the potential role of metapopulation structure in shaping phenotypic plasticity (Sultan and Spencer, 2002). These studies have shown that a high migration rate should favor phenotypic plasticity but only if the environmental indices are reliable. A meta-analysis of marine animals confirms this

prediction by highlighting a positive correlation between the rate of dispersal and the degree of phenotypic plasticity (Hollander, 2008).

I-3.4. Physiological mechanisms involved in phenotypic plasticity

Research on the physiological mechanisms involved in certain process in animal have been extended during the past few years. Until recently, plasticity was viewed strictly as an interaction between a gene for plasticity and environmental variables. It is now clear that phenotypic plasticity is not only the result of an interaction between environmental factors and a gene but, physiological mechanisms generating different morphs are also involved. What we observe as a phenotypic plasticity is in fact the plasticity of a broad diversity of developmental processes that underlie the phenotype (Nijhout, 2003a). Physiology and development provide the mechanisms through which genetic and environmental factors affect the expression of the phenotype. Understanding these mechanisms is crucial for the full perception of how phenotypic plasticity is generated in organisms and hence evolutionary biology (West-Eberhard, 2003). Phenotypic plasticity results from variation in developmental, physiological, biochemical, and behavioral processes that are sensitive to environmental variables. Most traits that have been of interest in the study of phenotypic plasticity (such as body size, fecundity, etc…) are established during post-embryonic development and are therefore unlikely to be directly influenced by the genetic processes that regulate early embryonic development (Nijhout, 2001).

An example of trait that can be used to show how physiology is involved in phenotypic plasticity is the variation of body size or growth rate in ectotherms. Body size and growth rate are both among the most common and widespread traits in evolution (Bradshaw and Hardwick 1989, Pigliucci, 2001). These traits can be indirectly affected by nutrition; in fact, tissues composing the bodies of insects do not respond directly to the circulating nutrients contained in the hemolymph. Although nutrients are necessary for growth, they do not appear to be an enough condition for growth. In most animals that have been investigated, the growth of cells and tissues is controlled by specific growth factors or growth hormones (Britton *et al*., 2002). These growth factors are generally neurosecretory hormones or hormones controlled by the central nervous system (Nijhout, 2003b). In such circumstances, plasticity can be affected by the degree to which environmental factors can affects these physiological events. In the tobacco hornworm *Manduca sexta* for example, nutrition and temperature can alter the growth rate and therefore the total mass that accumulates during the interval between the achievement of the critical weight and the final secretion of ecdysone. Plasticity

of body size in response to nutrition is mainly caused by the nutrition on the growth rate and on the value of the critical weight (Davidowitz and Nijhout, 2004). Temperature can also affect the rate at which the juvenile hormone breaks down, and therefore the total time available for growth. Accordingly, plasticity of body size in response to variation in temperature is because of temperature on both the overall growth rate, and on the delay time between the critical weight and the secretion of the Prothoracicotropic hormone (PTTH) and ecdysone (Davidowitz and Nijhout, 2004).

I-3.5. Importance of phenotypic plasticity

Given the current biodiversity of living organisms, their geographical distribution on earth and modifications that their living environment can undergo, one can immediately understand why phenotypic plasticity is important. Indeed, these organisms live in variable environments, sometimes imposing deep changes in their behavior and physiology to ensure persistence in the same environment. The arguments below justify the importance of phenotypic plasticity:

- It helps organisms to cope with varying environment; this is one of the main reasons why phenotypic plasticity occurs in nature. In fact, it is known that environments in which organisms live are not constant entities; they usually undergo variations on its biotic and abiotic components and the magnitude of variation is directly linked to the ecosystem context;
- during the first half of the 20^{th} century, Mendelian genetics (mainly mutation process) was intensively explored and stated as the key factor of evolution process. However, an aspect of the evolution through mutation was ignored since natural selection does not operates among genotypes, but among phenotypes. Moreover, mutations are rare and mostly deleterious while a single environmental factor can alter the phenotypes of an entire population, providing natural selection with access to perhaps thousands of environmentally altered individuals, as opposed to a single mutant individual (West-Eberhard, 2003);
- phenotypic plasticity also has a practical importance in the sense that it helps correcting some mistakes that can arise in taxonomy; in fact, highly plastic genotypes have been sometimes considered as different species. This phenomenon is particularly problematic among insects where environmentally induced phenotypes are confused as distinct species (Brakefield and Larsen, 1984). This failure in identifying an insect can hamper basic research, disease diagnostic and medical and agricultural pest control.

I-3.6. Molecular basis of phenotypic plasticity

Several definitions of phenotypic plasticity gene have been given. The one proposed by Pigliucci (1996 and 2001) is: "the regulatory loci that directly responds to a specific environmental stimulus by triggering a specific series of morphogenic changes". According to this definition, phenotypic plasticity genes can be identified as all of those encoding receptors for the environment that result in phenotypic responses (e.g. by activation of developmental cascades via hormones). Examples of this type is not lacking in the literature: genes coding for photoreceptors, bacterial genes leading to the transcription of operons in certain environments, etc. These are not the only genes involved in the expression and evolution of phenotypic plasticity in nature. Any gene whose products affect phenotypes differentially in different environments contributes to phenotypic plasticity (Pigliucci, 1996).

Phenotypic plasticity can be considered as a sequence of steps from a particular environmental condition to a particular phenotype. The chain begins with an environmental stimulus, which must be detected by a receiver that will generate a signal which in turn may lead to other signals. They will then be passed on to be read and translated in a process that leads to the phenotype (Windig *et al*., 2004). Current studies on the molecular mechanisms of phenotypic plasticity attempt to determine the mechanisms that link changes in environmental conditions with changes in organ development that result in the production of different phenotypes (Beldade *et al*., 2011).

To the question of how the control is done and the implication of the genes is made, there are always opposing ideas. As such, Pigliucci (1996) suggested that phenotypic plasticity is usually under the control of only one or a few major genes, but Windig *et al*. (2004) suggested that many genes interact to produce a plastic response to the environment. Regardless of the allelic or genetic composition of an organism, changes induced by the environment during development will ultimately result in a change in gene expression. The best-known example of gene whose expression depends on the environment is that encoding heat-shock proteins (Hsp). Their expression is influenced by temperature or other types of environmental stresses in order to buffer disturbances during development and allow the production of phenotypes (Takahashi *et al*., 2010). New molecular tools such as microarrays and RNA-Seq now allow access to the entire transcriptome and more studies have shown differences in gene expression between different environments, for example, differences in gene expression of *Drosophila melanogaster* larvae raised at different temperatures that are correlated with adult weight (Bochdanovits, 2003).

Several mechanisms are known to affect gene expression. The role of Endocrine hormones and DNA methylation are among the most studied (Beldade *et al*., 2011). The role of hormonal regulation has been demonstrated in most examples of developmental plasticity (Nijhout, 2003a). In insects, juvenile hormone and ecdysone are involved in the well-known example of seasonal polyphenism in the *Bicyclus anynana* butterfly (Brakefield *et al*., 1998).

I-3.7. Graphic representation and modelling phenotypic plasticity: the reaction norm

The term reaction norm was created by Woltereck (1909) when he was studying the pattern variation of some traits in different populations of daphnia. Pigliucci (2001) defined it as the function that links the environment to which a genotype may be confronted and the phenotypes that may be produced by that genotype. In many cases, the reaction norm is simply represented by a straight line characterized by its slope and intercept. But, the graphic representation of reaction norm can show more complicated shapes. Phenotypic plasticity is a consequence or a characteristic of the reaction norm which is itself expressed as the slope of the reaction norm (Figure 2).

The different components of the reaction norm can also be modeled using the Analysis of Variance as follow: $\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{G*E} + \sigma^2_{err}$

Where:

- σ^2_P is the total phenotypic variance;
- σ^2_G is the proportion of the variance that can be attributed to the differences between genotypes;
- σ^2_E is the variance associated with environmental change;
- σ^2_{G*E} is the interaction term because genotypes do not respond all in the same way to the environment, this term is the most important since it corresponds to the quantification of the genetic variation of the phenotypic plasticity;
- σ^2_{err} is the residual variance that includes the experimental errors.

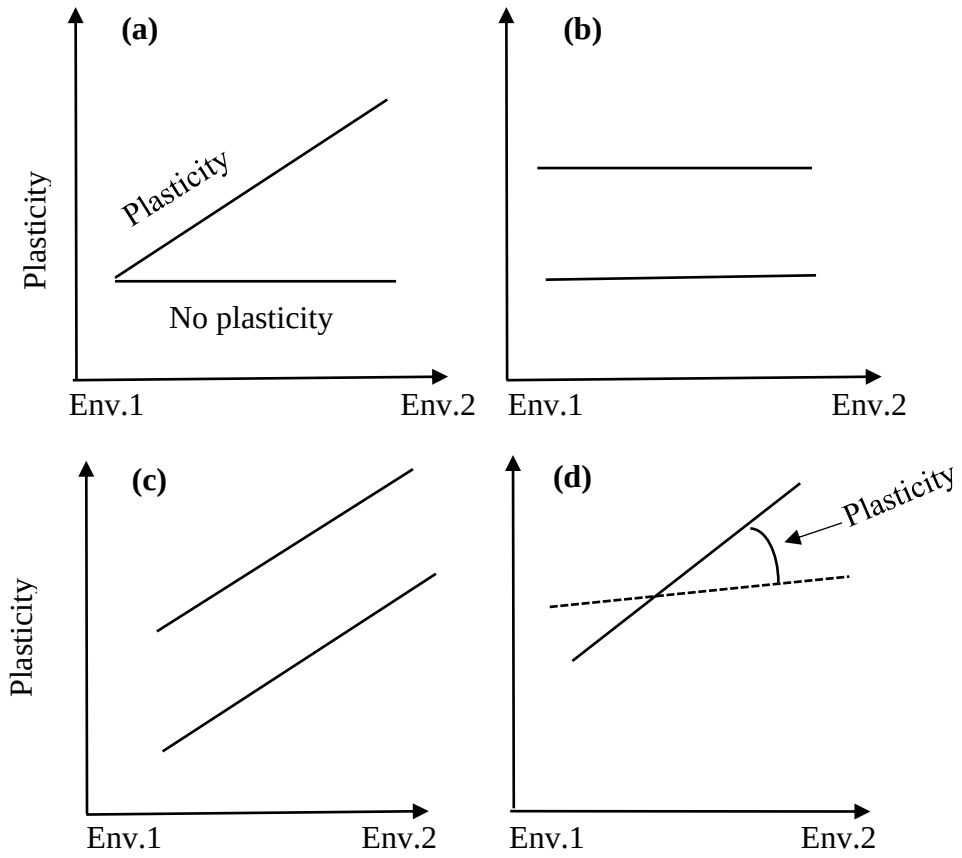

Figure 2. Representation of the relationship between the reaction norm (lines) and phenotypic plasticity, where reaction norms represents genotypes redrawn from Pigliucci (2001). (a) reaction norms indicating a plastic vs canalized response; (b) no genetic variation, no variation for plasticity; (c) plasticity, no variation for plasticity; (d) plasticity, variation for plasticity.

I-3.8. Special case of phenotypic plasticity: polyphenism

In 1963, Mayr suggested the use of the term polyphenism to make a distinction with another related term, polymorphism which connotes the genetic diversity in different populations or species. He stated: "*In order to make the term 'polymorphism' more useful and precise, there is now a tendency to restrict it to genetic polymorphism. Since this would leave nongenetic variation of the phenotype without a designation, the term 'polyphenism' is here proposed for it…".* Polyphenism is a case of phenotypic plasticity where individuals can express two or more discrete, discontinuous alternative phenotypes in different developmental environments (Nijhout, 2003a; Simpson *et al*., 2011). It can be induced by diet, population density, weather conditions, the presence of a member of a particular sex, or by the presence of a predator. It is a mechanism used by many organisms to escape from harsh conditions in the nature like unfavorable seasons, predator's avoidance, and reproduction

pattern (Roskam and Brakefield, 1999; Gilbert, 2002; Lyytinen and Brakefield, 2004). That is the main reason why it is generally interpreted as being adaptive by many authors because the maintenance of such distinct forms in a population suggests fitness benefits for the expression of these phenotypes in a specific environment (Brakefield and Zwaan, 2011). Alternative phenotypes are produced under the control of environmental cues acting at the early developmental stages of ontogeny. Most of the time, polyphenic traits are expressed at the adult stage, suggesting that the environment to which the alternative phenotype is an adaptation is not the same as the environment that induces the development of that phenotype (Nijhout, 2003a). Although the morphological discontinuity of some polyphenisms is produced by discrete developmental switches, most polyphenisms are due to discontinuities in the environment that induce only portions of what is a continuous reaction norm.

Many aspects of polyphenisms have been studied in numerous insect taxa. Among insects, Lepidoptera have been successfully used more than any other group regarding ecological conditions favoring the evolution and maintenance of polyphenism. Such studies have been very informative in the understanding of morph determination in these insects by using different approaches. Thus, experiments conducted in the laboratories and in natural conditions using Lepidoptera as model species helped to understand how ecological conditions, genetics and development shape the evolution of polyphenic systems (Wijngaarden and Brakefield, 2000; Beldade and Brakefield, 2002). Moreover, approaches with comparative methodologies may offer insight into the general roles of developmental mechanisms in the evolution of polyphenism specifically, and perhaps more generally, into the roles of development in shaping the evolution and diversification of morphology and life history, and ultimately new species (West-Eberhard, 2003).

What makes polyphenism a key solution used by ectotherms in coping with changing habitat is its success in the process of evolution of fitness in seasonal environments. Polyphenism allows different morphs of an ectotherm to survive in seasonal habitat with each form having higher fitness during the season in which they can be found. This form of polyphenism is known as seasonal polyphenism and it is used by many insects' species (Shapiro, 1976; Brakefield and Larsen, 1984). An example providing strong evidence of seasonal differences in fitness between morphs and insights about the reasons why such differences exist is that of the variation in wing pattern elements and some behavioral aspects in the squinting bush brown, *Bicyclus anynama* (Butler, 1879) in eastern and southern Africa (Brakefield, 1997). This satyrid butterfly occurs during the wet and dry seasons in its habitat. During the wet season in Malawi, adults exhibit wings with large submarginal ventral

eyespots which, exposing at rest is probably used as deflector for potential vertebrate predators (Lyytinen and Brakefield, 2004). Moreover, wet season morphs are short-lived; they quickly reproduce on the luxuriant herbage during the warm rainy season. In contrast, dry season morph exhibit wings with reduced submarginal ventral eyespots. They are inactive for much of their adult life and appear well camouflaged when at rest with wings closed amongst dead brown leaf litter. They must survive several months before they can lay eggs at the beginning of the rainy season when larval food plants (grasses) produce fresh foliage (Roskam and Brakefield, 1999). Though it was thought and proved that temperature alone can induce seasonal polyphenism in *Bicyclus* butterflies (Roskam and Brakefield, 1996), in nature it is more like a combination of many factors among which developmental time seems to be the main cue (Brakefield *et al*., 2007). Moreover, rainfall and food quality are also important factors that can have an impact in inducing any form of plasticity in *Bicyclus* butterflies (Kooi, 1992; Windig *et al*., 1994; Kooi *et al*., 1996; Kooi and Brakefield, 1999).

I-4. Thermal regulation in insects

I-4.1. Temperature: an important factor in ectotherms' physiology

Temperature is the main factor of the environment responsible for climates and hence the composition of terrestrial ecological communities (Cossins and Bowler, 1987). It constrains the rates of chemical and biochemical reactions. It is particularly crucial for ectotherms because the lack of thermal regulation combined with their relatively small size, force them to tolerate ambient temperature which, in the absence of thermoregulatory behavior, has a direct impact on their body temperature and hence their distribution (Kingsolver and Huey, 2008). This is especially true for insects which because of their generally short development time, will have several generations during the season under different climatic conditions, making them more sensitive to temperature variations.

I-4.2. Thermal sensitivity

Thermal sensitivity is not the same among different group of organisms or even to different populations of the same species found in different environmental conditions. This is because thermal sensitivity is function of an array of factors that structure the physiology of heat (Huey and Stevenson, 1979). Some organisms can tolerate very low (-5ºC) to very high (110ºC) temperatures while others live in a very narrow thermal range. However, some organisms can tolerate temperatures outside these ranges, but their physiological properties will not function normally during exposure to such extremes. That is why the present distribution of living organisms is highly correlated with regional

temperatures which acts as the major abiotic factor determining the presence or the absence of a given species at a given area (Sunday *et al*., 2012; Kaspari *et al*., 2015).

If the interest is the range of temperatures that an individual can tolerate and its most favorable thermal conditions, thermal performance curves (i.e. reaction norms linking the value of fitness-related traits, such as locomotion, assimilation, growth, development, fertility and survival) are appropriate and widely used methods (Huey and Stevenson, 1979). These curves typically reveal a biphasic effect of temperature, where in a first phase the estimate of the performance increases, then in a second phase decreases quickly with increasing temperature, resulting in a "bell" curve generally asymmetrical (Figure 3). These performance curves have proven to be an effective tool to understand the evolution of the thermal sensitivity of ectotherms as well as patterns of spatial distribution of individuals and the impact of climate change. The construction of such curves clearly demonstrates that any activity has a temperature at which performance is optimal (optimum body temperature). When given a choice, insects and other poikilotherms readily select temperature conditions that will maximize their performance (Walther *et al*., 2002; Kingsolver *et al*., 2011). For a single individual the parameters of the thermal performance curve may vary for different activities, and like other characteristics, the shape, height, and limits of the curve are subject to change through natural or artificial selection.

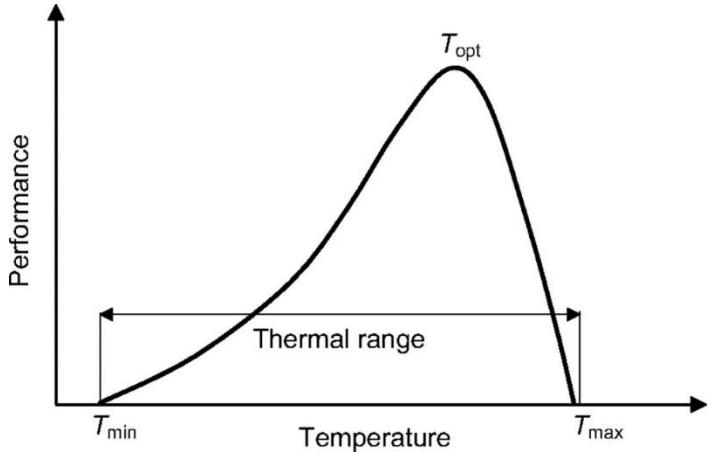

Figure 3. Hypothetical thermal performance curve with the stereotypical optimum at an intermediate temperature. The thermal optimum (Topt), performance breadth, critical thermal limits (CTmin and CTmax), and maximal performance (Pmax) are labeled. Adapted from Huey and Stevenson (1979).

I-4.3. Ecological responses of insects to recent climate change

Global warming is indicated by the average increase in temperature at the surface of the earth and oceans since the mid-twentieth century. Surface temperature has increased by 0.74 °C and is projected to increase by 1.1 to 6.4°C during the 21st century (Pachauri *et al*., 2015). Moreover, a very likely rise in high temperatures, heat waves and episodes of heavy rainfall is also forecast. There is nowadays ample evidence that recent climate change is affecting biodiversity with important consequences including local extinctions, population shifts, shifts in community structure and composition, and changes in phenology (Parmesan, 1996; Easterling *et al*., 2000; Thomas *et al*., 2004). In the face of these environmental changes, a major preoccupation is to better understand the response mechanisms that species, especially ectotherms are going to implement. Basically, there are two main responses used by ectotherms to cope with recent global warming: either a modification of the genetics composition of the population, the most successful genotypes specialized in a given environment will be selected by natural selection (Hoffmann *et al*., 2003) or an adaptation in the middle by phenotypic plasticity, whether at the level of individuals (e.g. behavioral modifications) or at the level of the offspring (developmental plasticity). A combination of these two answers is possible and even probable (Ghalambor *et al*., 2007).

Among these responses, the simplest way to challenge climatic changes by ectotherms is the modification of the phenology of organisms because it is highly correlated with climatic factors (Chuine 2010). Phenological modifications due to climatic changes can drive the movement of many species. For example, evidence showing modification on the phenology or organism can be early appearance of butterflies, early arrival of migratory birds and early flowering of plants.

The relative importance of these responses will depend on different factors: the importance of environmental change and the time scale considered, the life history traits of the species, the availability of alternative habitats, and the dispersal capacity of the species (Gienapp *et al*., 2007). Many studies on climate change responses are only phenotypic and do not distinguish between phenotypic plasticity and genetic response. Although recent examples from the literature show that a rapid genetic response (in a few generations) to climate change can be put in place (Balanya, 2006; Bradshaw and Holzapfel, 2008; Gienapp *et al*., 2008), allowing species to react in a very short time scale (intra-generation), there will be a need of time to implement changes and the selection of traits that can increase fitness of the populations exposed.

CHAPTER II:

MATERIAL AND METHODOLOGY

II-1. Study sites

II-1.1. General characteristics of physical environments in Cameroon

II-1.1.1. Geographic location

Cameroon is a country straddling West and Central Africa and lies between latitudes 1º and 13º N, and longitudes 8º and 17º E. It extends over nearly 12 degrees of latitude and 9 of longitude for an area of approximately 475000Km^2 (Mulua and Lambi, 2006). The country's neighbors are Nigeria in the west, Chad to the northeast, the Central African Republic to the east, and Equatorial Guinea, Gabon and the Republic of Congo to the South. Often called "Africa in miniature", Cameroon has one of the most complex and diverse topographic, vegetation and climate in Africa.

II-1.1.2. Relief

The relief of Cameroon is extremely variable, partly due to the presence its volcanic line and the Adamaoua plateau. The volcanic line of Cameroon begins in Atlantic Ocean at the level of Sao Tome and Principe and the Bioko Islands. It is oriented in a SW-NE direction and consists of several important mountains like Mount Cameroon (4,100 m), Mount Manengouba (2,411 m), Mount Bamboutos (2,679 m), Mount Oku (3,011 m), Mount Koupé (2,064 m), Mount Bana (2,045 m), and Mount Nlonako (1,822 m) (Neba, 1999). These mountains are dotted by a series of mountain ranges that continues practically up to the level of the Mandara Mountains, intersecting with the Adamaoua plateau at the Tchabal Mbabo complex. The Adamaoua plateau is a large block of raised base punctuated by small volcanoes with pics at 2,000 m. The North Cameroon (North to the Adamaoua plateau) is made up of three dominant orographic units, namely: (1) the depression of Lake Chad consisting of the plains such as Logone and Diamaré, (2) the Benue basin dotted with some few mountains, and (3) the Mandara mountains, which is the upland zone formed by a group of mountains, the most important of which are Mount Tourou and Rhumsiki. In the south, Adamaoua plateau is abruptly connected to the Adamaoua falls and descends towards the South-Cameroon plateau. The southern Cameroonian plateau, with an altitude varying between 650 and 900 meters, covers about one third of the country's area, from the Adamaoua falls to the southern border and borders west by the western plateau. The coastal plains extend along the Nigerian border to the north-west (Mamfe basin), narrowing on the outskirts of Mount Cameroon, widening in the sedimentary basin of Douala and extend up to the border of Equatorial Guinea (Billard, 1962).

II-1.1.3. Vegetation

Cameroon's vegetation is often considered as a digest of that of the African continent for several reasons. Indeed, there is an equatorial dense humid rainforest, central savannas and northern steppes. In addition to these large complexes, there are also particular vegetations that are related to topographical, hydrographic, edaphic, climatic or anthropogenic impacts. Although the vegetation of Cameroon as described by Letouzey (1985) and Servant and Servant (2000) has experienced deep modifications, it was used simply because there are no other recently published documents that describes the vegetation of Cameroon with such details. However, the vegetation described here considers the profound changes that are perceptible nowadays (Djoufack, 2011).

In general, Cameroon has five agroecological zones that cover the ten administrative regions: (1) the Sudano Sahelian zone, (2) the high Guinea savanna, (3) the western highlands, (4) humid forest with monomodal rainfall, and (5) humid forest with bimodal rainfall (IRAD, unpublished data). Cameroon's natural environment has a physiography that is influenced anthropogenic activities. The Southern plateau is mainly dominated by tropical dense forests which have been considerably degraded due to human activities although there are still weakly distributed primary and secondary forests patches, particularly in some localities in the South eastern regions. In the Atlantic coastal area, from Bakassi to Kribi, there are both mangroves and lowland evergreen forests whose altitude rarely reaches 150 m. Behind this mangrove, another evergreen rainforest can be found which lies within the Mamfé basin. In the western highlands, landscape has been deeply shaped by agricultural activities, with industrial plantations. This zone is occupied mainly by crop land and planted rubber and palm trees. In addition to these large complexes, there are also particular natural forest types namely sub-montane and montane respectively above 1,200 and 2,200 m that can be found on the slop of Mount Cameroon, Mont Oku and several others mounts. Above 3,200 m are alpine forests. The forest-savanna interface zone in Cameroon corresponds to a wide area between the fourth and the sixth degrees of north latitude, and between the eleventh and the sixteenth degrees of longitude. This type of vegetation is perceptible in localities such as Yoko, Ndikiniméki, Tibati, Bétaré Oya (Servant and Servant, 2000).

The Adamaoua plateau has buffer vegetation between the forest to the south and the steppe to the north, but this vegetation is gradually altered and converted to grassy savanna in the northern plain. The vegetation cover in these savannas is discontinuous and composed of some areas of grasses based on *Hyparrhenia* spp., while in others *Panicum* spp. and *Sporobolus* spp. are required. This grassy vegetation is being altered by the advance of the phenomenon of desertification and the bush

fire generated by farmers. In the North, the Sudanian zone includes wooded savannas, dry forests of the Benue Basin and Sudanese highland formations on the Mandara mountains with high human population density, and consequently are intensely cultivated. In the extreme north, the Sahelo-Sudanese zone includes thorny steppes and periodically flooded grasslands and Acacias abound.

II-1.1.4. Climate

In general, there are two climatic trends in Cameroon: equatorial climate in the south and tropical one in the north of the Adamaoua plateau.

The southern part of the country (which lies around latitude 2 to 6 degrees north) is dominated by an equatorial climate which itself has two variants:

- the Guinean type extending from the coast of Kribi and covers the southern plateau. It is characterized by four yearly seasons per year (02 rainy and 02 dry), with annual rainfall ranging between ~1,500 and ~2,000 mm and temperatures varying from ~22 to ~35ºC (IITA, unpublished data);
- the mountain type prevails in western highlands and is characterized by two seasons, a long wet and a short dry season; temperatures vary from 12 to 36ºC with annual rainfall oscillating between 1,000 and 1,500 mm;
- the maritime influenced climate is mainly present in lowland coastal zone where temperature, relative humidity and rainfall are very high. This section encompasses the second rainiest location of the world, Debundscha, with rainfall amount of up to 11,000 mm per year (Mulua and Lambi, 2006).

The tropical climate domain extends from latitude 6ºN to 13ºN and is subdivided into three main sub-categories:

- the humid tropical highlands climate dominates the Adamaoua plateau and is characterized by rainfall reaching 1500 mm per year, average temperature of 21ºC. There are two distinct seasons: the wet season which lasts for over seven months while the dry season, sometime harsh, lasts between 3 to 4 months (Mulua and Lambi, 2006);
- the Sudanese type covers the Benoué basin, characterized by rainfall reaching 1,300 mm per year, average temperatures of 28ºC. There are two seasons, the wet and the dry which last six months each;
- the Sudano-sahalian climate is mostly found in the far north of Cameroon where average temperature is about 28.5ºC and rainfall is about 800 mm per year. In certain localities it

rains only 400 mm a year for four months while the intense dry seasons extends for eight months.

II.1.2. Sampling localities and their characteristics

Data collection was conducted in the four locality types (Figure 4). These localities were selected based on the vegetation and prevailing climate. They are found either in the forest (Mbalmayo and Somalomo) landscape or in the forest-savanna transition zones (Ndikiniméki and Ako), much better known as ecotones (Smith *et al*., 1997).

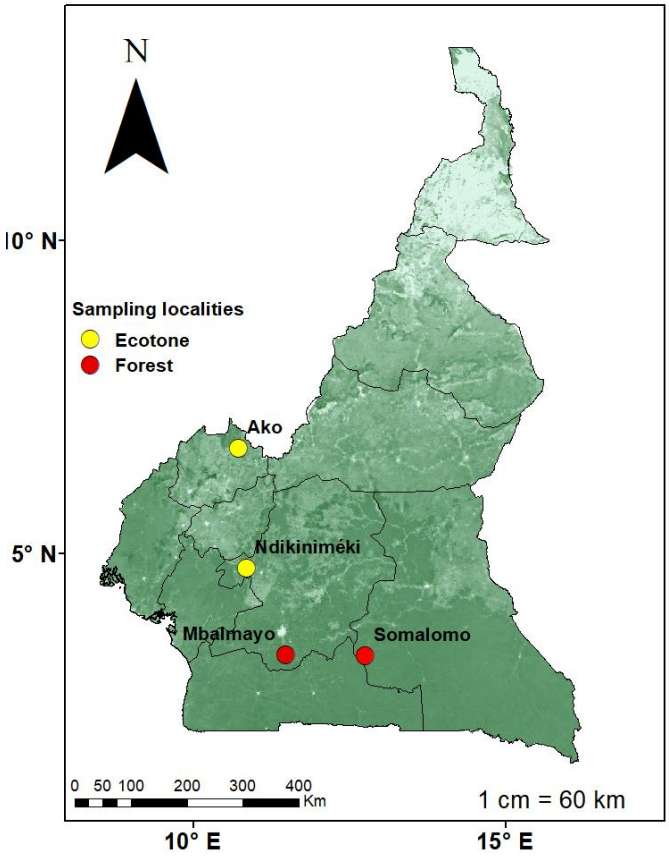

Figure 4. Map showing sampling sites of *B. dorothea* in Cameroon. Yellow circles represent ecotone sites and green ones represent forest (Map by Dongmo).

II.1.2.1. Mbalmayo

The Mbalmayo location is situated in the southern plateau, precisely in the Center region of Cameroon, about 50 km south east to Yaoundé. Our sampling was conducted in a site (N: 3.388, E: 11.47, 750 m a.s.l) located 7 km from the main town. Vegetation is dominated by humid forest, that

has been degraded by human activities principally agriculture. Mbalmayo is subjected to a transitional equatorial climate with 04 seasons (02 wet and 02 dry); average monthly temperatures range from 22 to 25ºC, with relative humidity varying with the seasons (typically 60 – 90% RH); rainfall is bimodal and can reach 2,000 mm yearly (IITA, unpublished results). The ombrothermic diagram of the site is given in Figure 5.

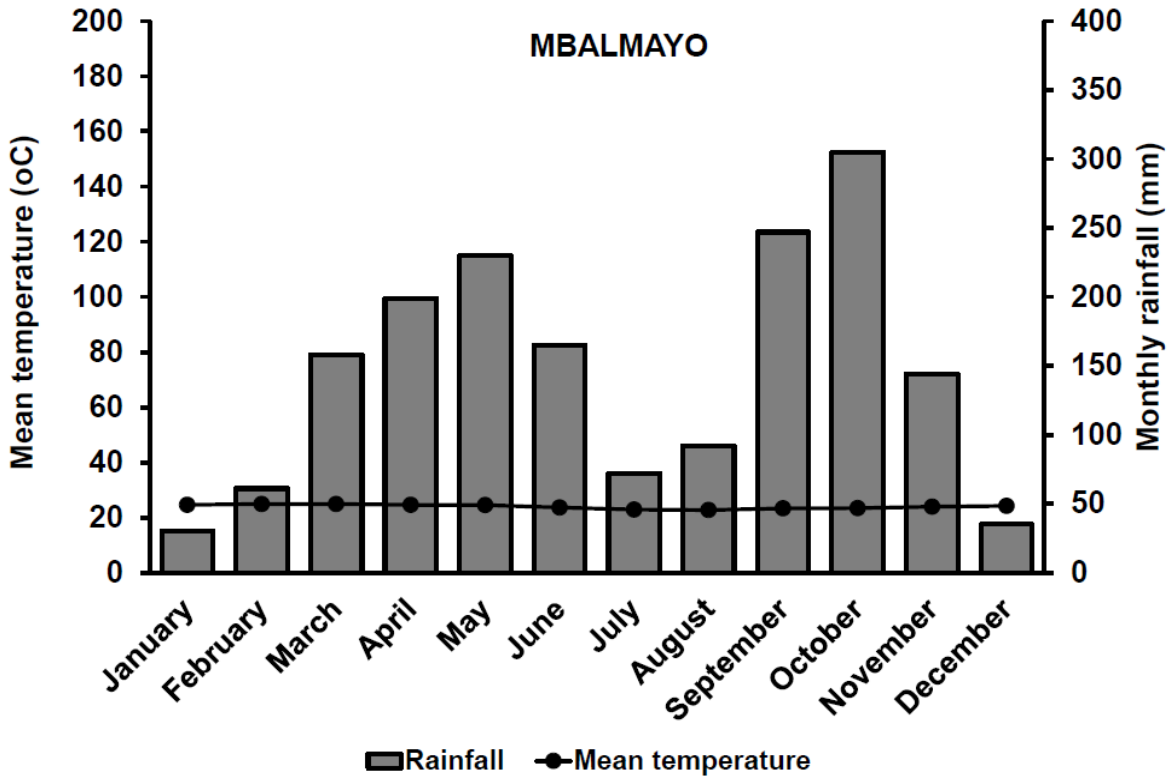

Figure 5. Pattern variation of monthly mean temperatures and total monthly rainfalls of the locality of Mbalmayo from January to December 2016 (IITA, unpublished results)

II.1.2.2. Somalomo

Somalomo is the main town situated at the northern western antenna of the Dja Faunal Reserve. The buffer zone of the Dja reserve is occupied by villages. The distance between the effective reserve and villages ranges between 7 to 15 km. Due to the presence of personnel from the Cameroon Ministry of Forest and Wildlife, human activities in the buffer zone are regulated to reduce their pressure on the Dja Reserve. To that end, the sampling site were located 20 km from Somalomo town where secondary forest could be found. The site's is at 600 m a.s.l and is characterized by an equatorial climate with 04 seasons (02 wet and 02 dry), a bimodal rainfall regime of up to 2,000 mm

per year, average monthly temperature ranging from 22 to 25ºC and relative humidity ranging 70 to 95% (Figure 6).

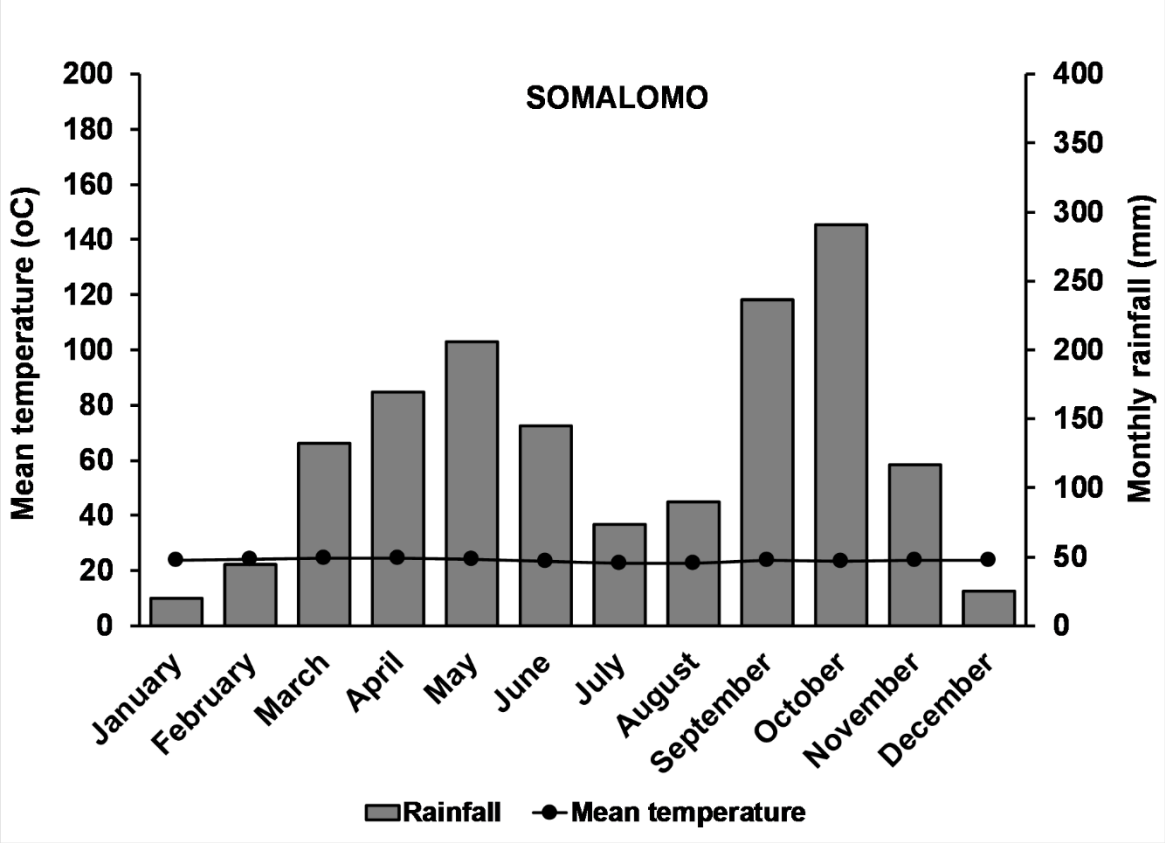

Figure 6. Pattern variation of mean monthly temperatures and total monthly rainfalls of the locality of Somalomo from January to December 2016 (IITA, unpublished results).

II.1.2.3. Ndikiniméki

The locality of Ndikiniméki is located in the depression between the western highlands in the west, the southern plateau, and the coastal plain in the south of Cameroon. Average altitude is about 800 m a.s.l with bimodal rainfall pattern (about 1,700 mm per year) (Tsalefac *et al.*, 2003), a vegetation composed of a mosaic of gallery forests and savannas, average monthly temperature varying from 22 to 25ºC and relative humidity of 50 to 80% (Figure 7).

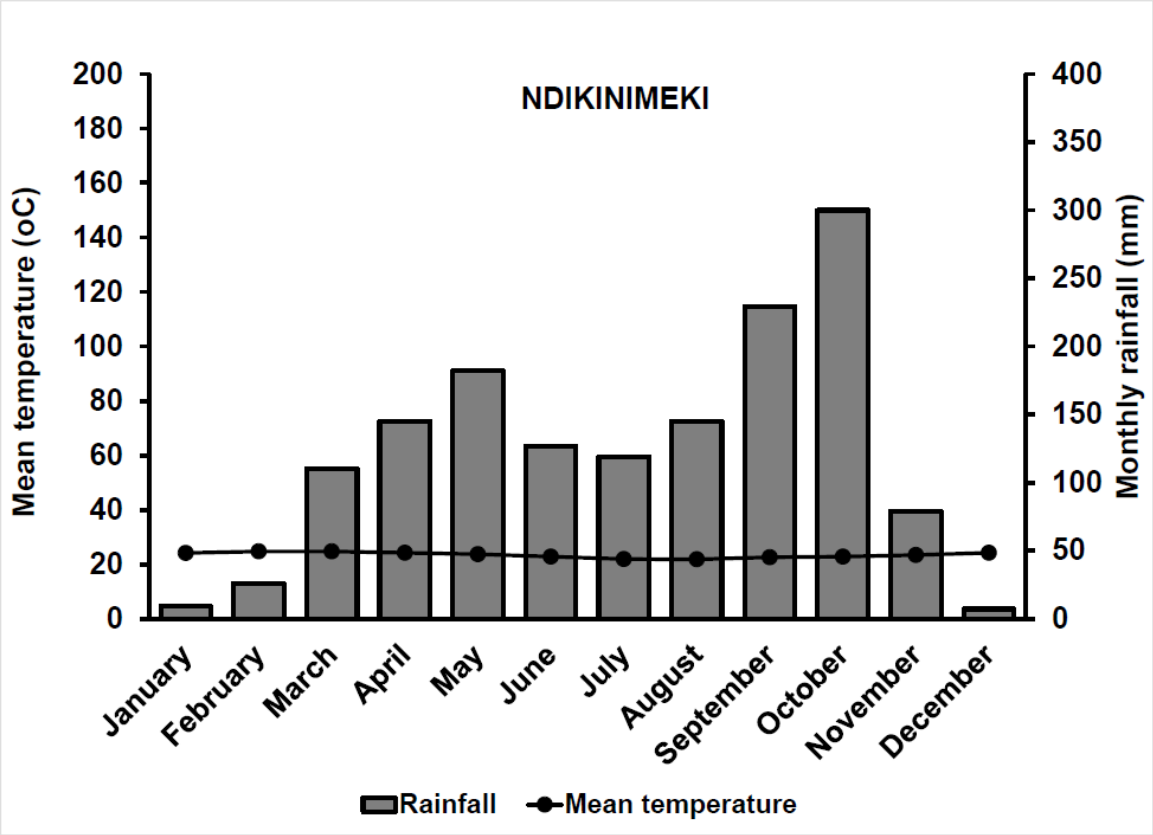

Figure 7. Pattern variation of mean monthly temperatures and total monthly rainfalls of the locality of Ndikiniméki from January to December 2016 (IITA, unpublished results).

II.1.2.4. Ako

Ako is a subdivision located in the North West region near the Cameroon-Nigeria. It is also located between the highlands of North-West Cameroon and the East-Nigerian plain with and altitude between 350 and 400 masl. Rainfall is unimodal and the dominant vegetation is a mosaic of sub-montane forest and upland savannah. Mean monthly temperatures vary between 23 to 27ºC while relative humidity ranges between 50 and 85% and about 1500 mm annual rainfall (Figure 8).

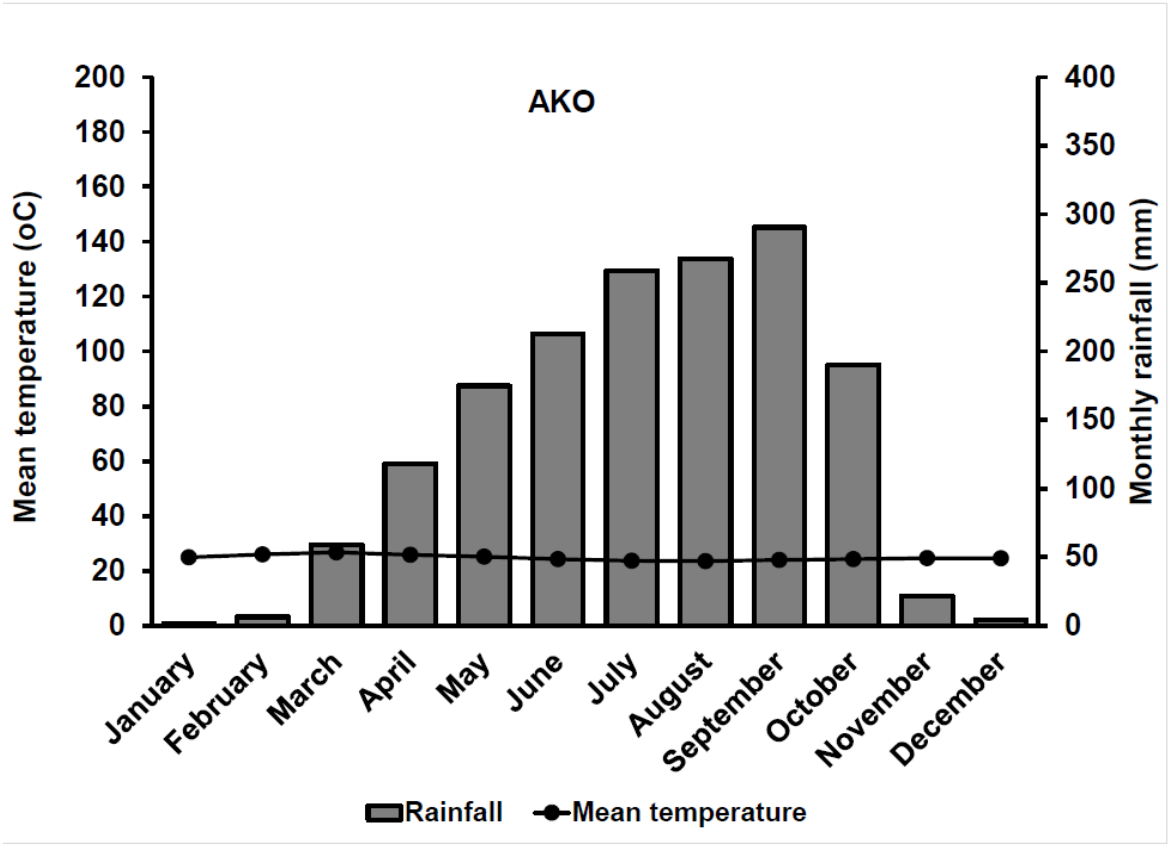

Figure 8. Pattern variation of mean monthly temperatures and total monthly rainfalls of the locality of Ako from January to December 2016 (IITA, unpublished results).

II.2. Material

II.2.1. Biological material

The biological material was made of animal and plant materials. Animal material was represented by adult butterflies captured at each of the study sites mentioned above; immature stages (eggs, larva, pre-pupa and pupa) were also used during experiments. The grass *Axonopus compressus* P. Beauv., 1812 and millet *Pinnesetum glaucum* (L.) R. Br. 1810 represented plant materials which were respectively used as larval host plants and egg laying host plants (Figure 9).

II.2.2. Technical material

Technical material was represented by the following items: a conical fruit-baited trap, a sweep net, cubic cages (24×24×24cm), an environmental chamber (Percival, model I-36VL), dissecting set, alcohol 70º, high precision Jewelry GEM50 scale, a microscope equipped with a micrometer, and an electronic digital caliper (0 – 150mm/6inch).

Figure 9. Potted millet *Pennisetum glaucum* used as egg laying host plant for *Bicyclus dorothea* during laboratory experiments (Photo by Dongmo).

II.3. Methodology

II.3.1. Bio-ecological aspects of *Bicyclus dorothea*

II.3.1.1. Developmental stages

To assess and describe the developmental stages of *B. dorothea,* individuals were captured at Mbalmayo using overripe banana-baited traps (Figure 10a) while hand-netted captures were also conducted. Captured individuals were brought to the laboratory of entomology of IITA, Yaoundé and kept in cages (24 × 24 × 24 cm) (Figure 10b) made of white polyester screen (to facilitate insect activities and good ventilation) containing mashed banana and water-soaked cotton (Brakefield *et al.*, 2009). Potted young millets plants (*Pennisetum glaucum*) were added to the cages on which *B. dorothea* may laid its eggs. Eggs laid daily were carefully transferred with a moistened camel hair brush to Petri dishes lined with moistened black filter paper and exposed for eclosion in a room with a constant temperature of 26 ± 0.5ºC, a relative humidity ranging between 70 and 80% and a D12:L12 photoperiod. Satyrines feed on wild grasses belonging to the family Poaceae (Condamin, 1973). The lawn grass *Axonopus compressus* was used during this study as host plant for larvae. A total of 200 eggs were collected for this experiment. After eggs hatch, batches of ten newly hatched larvae were carefully removed and placed individually on potted lawn grass.

Figure 10. Set up for butterfly trapping and rearing: a) Conical fruit-baited trap used to trap butterflies; b) cage used to keep adult butterflies for reproduction (egg laying) (Photo by Dongmo).

Molting in Satyrines, as in other lepidopteran larvae, generally follows when the larva head capsule is removed (Condamin, 1973), and when there is a reduction of food intake and larval activity. Larvae were checked daily to detect possible molts corresponding to shift from one larval instar to another. The number of larval instars could be then calculated following Carey (1993):

N = n + 1

With N = Number of larval instars; n = numbers of molts.

Individuals were reared from eggs to adult stage; after imaginal molt, the adults were placed together (males and females) in cages for mating after which each female was isolated in other cage with a potted young millet plant as egg laying substrate. The survival rate and developmental time for each immature stage was estimated. Other parameters including adult fecundity and survival were also determined.

II.3.1.2. Morphological characteristics of the adults

In order to better describe the morphological features of *B. dorothea*, a sampling was conducted at Mbalmayo during the year 2015; wild individuals captured were stored in glassine envelopes and transported to the laboratory. A total of 140 butterflies (85 males and 54 females) were used to evaluate 13 morphological parameters (Figure 11). Morphometric characters were measured

using either a caliper (0.1 mm accuracy) on each specimen or a stereomicroscope fitted with a micrometer eyepiece at 6X magnification.

Figure 11. Characters measured by the stereo microscope and caliper. Shown are: (a) one fore and (b) one hind wing of *B. dorothea* male. d_1= length of the fore wing; d_2= width of the fore wing; d3= diameter of the first eyespot of the fore wing; d_4= diameter of the second eyespot of the fore wing; d_5= length of the hind wing; d_6= width of the hind wing; d_7= diameter of the first eyespot of the hind wing; d_8= diameter of the second eyespot of the hind wing; d_9= diameter of the third eyespot of the hind wing; d_{10}= diameter of the fourth eyespot of the hind wing; d_{11}= diameter of the fifth eyespot of the hind wing; d_{12}= diameter of the sixth eyespot of the hind wing; d_{13}= diameter of the seventh eyespot of the hind wing (Roskam and Brakefield, 1996).

II.3.1.3. Geographic distribution in Cameroon

A survey was conducted from April to October 2015 in seven regions of Cameroon (Adamaoua, West, North West, South West, East, South, and Center) to assess the occurrence of *B. dorothea*. We recorded the presence/absence data in each locality visited during the survey as well as other parameters such as the geographic coordinates (latitude, longitude and altitude).

II.3.2. Seasonal variation in wing pattern elements in *B. dorothea*

II.3.2.1. Butterflies' sampling

At each locality (see Figure 4), individual butterflies were caught using overripe banana-baited traps placed at appropriate sites in addition to hand-netted captures with a butterfly net. Sampling was conducted two or three times during the dry (trapping from January to March) and wet (trapping from June to August) seasons in both 2015 and 2016 at each locality. Captured butterflies were brought to the laboratory of entomology of the International Institute of Tropical Agriculture (IITA-Yaoundé, Cameroon), and kept in a room maintained at 26°C, 75% RH and D12:L12

photoperiod, and used for rearing study (for temperature-induced plasticity and thermal stress adaptation experiments). Butterflies were kept in cages (24 × 24 × 24 cm), made of white polyester mesh (to facilitate insect activities and good ventilation) containing mashed banana and water-soaked cotton (Brakefield *et al.,* 2009). After their death, wild-caught individuals were stored in small glassine envelopes for further morphological analysis.

II.3.2.2. Morphological measurement

Bicyclus dorothea's eyespots are similar in structure and position as in all *Bicyclus* species; they are located on the ventral side of the wings and are not visible from the dorsal side. There are nine spots in total, two on the forewings and seven on the hindwings; however, some individuals possess three spots on the forewing and eight on the hindwing. Eyespots are approximately circular in shape and made of numerous rings: a white ring in the center of the eyespot, a black disc, a cream yellow disc and an outer gold disc (Brakefield and French, 1997). Thirteen wing pattern characters (Figure 11) were measured on each individual. Since the spots are not perfectly circular, all measurements were done parallel to the vein of the wing. Moreover, measurements were conducted only on the left fore and hindwings. The right wings were used in case the left wings were damaged. To measure the diameter of the eyespot, the fore- and hindwings were placed between two glass slides under a stereomicroscope fitted with a micrometer eyepiece at 6X magnification. Wing length, from the thorax to the apex and the width of the fore and hindwings were measured using a caliper (0.1 mm accuracy).

II.3.3. Temperature-induced plasticity in *Bicyclus dorothea*

II.3.3.1. Culture and maintenance of different populations of *Bicyclus dorothea* in the laboratory

Adults *Bicyclus dorothea* individuals collected during the wet season at each locality (Ako, Ndikiniméki, Mbalmayo and Somalomo) could lay eggs from which hatchlings were further reared on natural lawn *Axonopus compressus* as host plant. Rearing occured in a room where the temperature was maintained constant 26 ± 0.5ºC, relative humidity ranging between 70 and 80% and D12:L12 photoperiod. To avoid the maternal effect (Mousseau and Dingle, 1991; Jenkins and Hoffmann, 1994; Mousseau, 1998), adults obtained from the first generation were allowed to mate in cages and resulted eggs (second generation) were used for the experiment.

II.3.3.2. Experimental design and measurement of plasticity

II.3.3.2.1. Development and wing pattern plasticity

We assessed development and wing pattern plasticity by rearing eggs collected from females (of all populations) of the first generation to the adult stage in incubators set at three different temperature regimes (22, 26 and 30ºC), relative humidity ranging between 70 and 80% and D12:L12 photoperiod. For each population, at least three batches of 100 eggs were lined on moist black filter paper in Petri dishes and exposed in a climate cabinet (Percival, model I-36VL) set at a given temperature with 75 to 80% RH and a photoperiod of D12:L12. Newly hatch larvae were counted and immediately transferred on potted lawn grass placed in a climate cabinet set at the temperature cited above. These hatchlings were allowed to develop to adult stage in each climate cabinet. Host plants were monitored daily and watered or replaced as necessary. Pre-pupae were collected daily and placed in Petri dishes to pupate. One-day-old chrysalids were weighed to the nearest 0.001 mg using a high precision Jewelry GEM50 scale, and then placed in individual 20ml-cups in their respective rearing temperature until eclosion. Egg, larval and chrysalid development times were recorded in days.

II.3.3.2.2. Measurement of wing pattern variation

Adult butterflies from previous rearing at each temperature were frozen at -40ºC and their wings were separated from the body for morphological measurements. Thirteen wing pattern characters (see Figure 11) were measured. Since the spots are not perfectly circular, all measurements were done parallel to the vein of the wing. To measure the diameter of the eyespot, the fore and hind wings were placed between two glass slides under a stereomicroscope fitted with a micrometer eyepiece at 6X magnification. Wing length, from the thorax to the apex and the width of the fore and hindwings were measured using a caliper (0.1 mm accuracy).

II.3.4. Thermal tolerance in *Bicyclus dorothea*

II.3.4.1. *Bicyclus dorothea*'s rearing

Bicyclus dorothea's eggs were collected from adult females sampled in each locality (Ako, Ndikiniméki, Mbalmayo and Somalomo) and kept in an insectarium maintained at 26ºC, relative humidity 75%, photoperiod L12:D12. These eggs were kept in Petri dishes lined with moistened black filter paper to allow them to hatch. Hatchlings were reared on population cages (100 ×50 × 100 cm)

and fed on potted lawn grass *Axonopus compressus* ad libitum. Pupae were collected daily and transferred to small cubic cages (10 × 10 × 10 cm); on their eclosion day, they were given mashed banana and cotton soaked with distilled water. They were maintained in the same insectarium with the conditions mentioned above during 24 hours before been used for all thermal tolerance experiments described below. To avoid maternal effects on heat resistance (Jenkins and Hoffmann, 1994), all adult butterflies used in this experiment were those from the second generation of the laboratory cohorts.

II.3.4.2. Determination of the critical thermal minimum (CTmin) and maximum (CTmax)

To measure the critical thermal minimum, one-day old adult butterflies were placed individually in small plastic bottles (250ml, see Figure 12a) perforated with about 25 holes (~3 mm diameter each) for aeration and were exposed in an environmental chamber (Percival, I-36VL) initially set at 25ºC. One hour after, the climate cabinet was set in a ramping mode with temperature decreasing at a rate of 0.5ºC per minute. An electronic thermometer (Figure 12b) was placed in the climate cabinet from which one could readily read temperature change in real time. The critical thermal minimum was the cold temperature at which adult butterflies were not able to make coordinated movements. For the critical thermal maximum, one-day old adult butterflies were also used and the same method as previous, but the climate cabinet was set in a ramping mode with increasing temperature at the same rate of 0.5ºC per minute. The critical thermal maximum was the high temperature at which each butterfly was not able to make coordinated movement as it was the case with the critical thermal minimum. We also assessed the thermal tolerance breadth of each population as a difference between the CTmax and CTmin (Huey and Stevenson, 1979). The number of individuals used at each experiment varied from 45 to 60 for both males and females.

II.3.4.3. Heat knock down time (HKDT)

This parameter is sometime used as a proxy to investigate environmental effects on temperature stress resistance; this index is considered reliable proxy of climatic heat adaptation (Sorensen *et al.,* 2005; Karl, 2008) and it represents the time required for an insect to enter an apparent 'coma' following exposure to high temperature (Huey and Stevenson, 1979). In most cases, it occurs when the insect is no longer able to stand on its feet or cannot have coordinated movement (Fischer *et al*., 2010). Butterflies from the second-generation colony established in the laboratory were used in this experiment. One-day old adults were kept individually into 250mL-small plastic bottles with

wholes (to allow air circulation inside the environmental chamber) and transferred into environmental chambers set at a constant temperature of 40°C (this temperature was chosen based on the highest temperature recorded in Ako (37°C) during the dry season); the environmental chambers were equipped with an external window allowing us easily watch the behavior of butterflies and to exactly know the time at which the HKDT occurred. The HKDT of each individual was recorded.

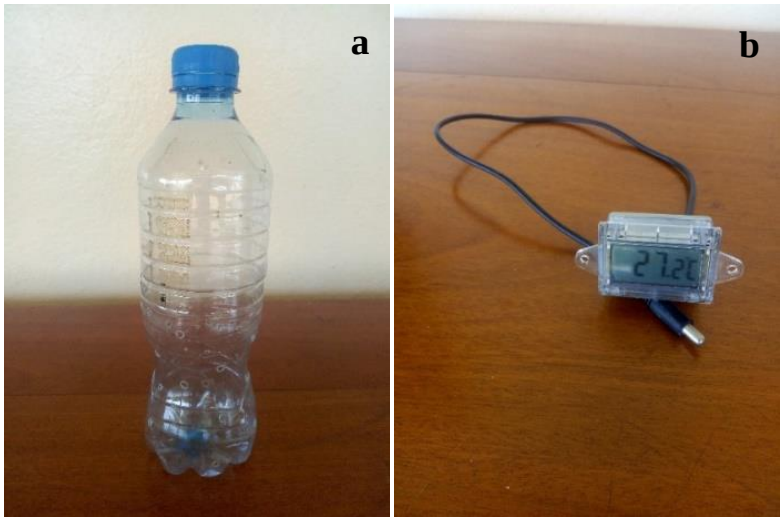

Figure 12. Set up for thermal tolerance assessment: a) Plastic bottles with holes used to assess the Ctmin and Ctmax for *B. dorothea* inside the environmental chamber; b) electronic thermometer used to assess real time temperature in the environmental chamber (Photo by Dongmo).

II.3.4.4. Heat knock down recovery time

After exposure to heat (40ºC), the heat knock down time was recorded and butterflies were immediately transferred to another incubator maintained at 25°C to record the recovery time corresponding to the moment where butterflies were knocked down and taken out from the incubator maintained at 40°C and the time when they stood on their legs within the following 120 minutes after which they were considered dead (Gibert and Huey, 2001).

II.3.5. Statistical analysis

II.3.5.1. Life history traits

The developmental time of all immature stages was estimated using the formula expressed as:

$\mathbf{D_k = \frac{1}{Nk} \sum (n_x \times X)}$ (Southwood, 1978) where:

(k = development stage; n_x = number of individuals moving from instar k to instar k+1 at the day X; **X** = number of days spent by individuals from instar k to move to instar k+1; $\mathbf{N_k}$ = total number of individuals moving from instar k to instar k+1;

The age-stage, two-sex life table approach made it possible to analyze the raw life-history data for *B. dorothea*. The survival rate of each immature stage was estimated as the probability of an individual of age *x* and stage *y* surviving to stage *j*. The age-stage-specific fecundity (f_{xj}) (the daily number of eggs laid by an individual at age *x* and stage *j*), the age-specific fecundity curve (m_x), the age-specific survival rate (l_x) (the probability that a newly laid egg will survive to age *x*), and the population parameters were calculated accordingly. The intrinsic rate of increase (r) was calculated with the Eule-Lotka equation as $\sum_{x=0}^{\infty} e^{-r(x+1)} l\text{xmx} = 1$. The *GRR* was calculated as $GRR = \sum m_x$. The finite rate of increase (λ) equaled e^r. Net reproductive rate (R_0) was measured as $\sum_{x=0}^{\infty} lx\text{mx}$. The mean generation time (T), defined as the time required for a population to increase to R_0-fold of its population size at the stable stage distribution, was calculated using the formula $T = (\ln R_0)/r$ (Carey, 1993). Finally, the means, standard errors and variances of the population parameters were estimated via the bootstrap technique using the TWOSEX-MS Chart program (Chi, 1988). The software R v 3.5.0 (Team, 2017) was used to create graphs. A two-sample student's t-test was used to assess significant differences among all morphological features in males and females.

II.3.5.2. Seasonal variation in wing pattern elements in *B. dorothea*

To analyze the morphological variation in wing characters, a principal component analysis (PCA) was performed on data of each sex to reduce the wing pattern variability for forest and ecotone habitats on the one hand and sampling localities on the other hand. The first two components were used for further analysis. The nested ANOVA model was applied on the two retained principal components with season, habitat and sex as fixed factors, and sampling sites nested in habitat. The models were constructed with interaction terms between seasons and habitat, and with sites nested in habitat. Visual inspection of residual plots of each model did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. All statistical operations were performed with R v 3.4.0 software (Team, 2017).

For each trait, the rate of change was estimated between wet and dry seasons for both males and females in each habitat using the following formula:

$$Rc = \left(\frac{Wf - Df}{Wf}\right) \times 100$$ (Marsden and Weinstein, 2012)

Where Rc is the rate of change of a given trait, *Wf* is the wet season form of the trait and *Df* the dry season form of the same trait.

A multivariate analysis of variance MANOVA was used to test the effect of sampling localities (fixed 4 levels: Ako, Ndikiniméki, Mbalmayo and Somalomo) and seasons (fixed, 2 levels: wet and dry) on the wing pattern elements. Normality was tested with Shapiro-Wilk test and homogeneity of variances with Levene's test. Data were log-transformed to fulfill MANOVA assumptions and to reduce the type I error. The Student-Newman and Keuls test was used when the probability value in the ANOVA was <0.05.

II.3.5.3. Data analysis for temperature-induced plasticity in *B. dorothea*

Before analysis, developmental times of all immature stages were log-transformed to make data fit the normal distribution. Two or three-way ANOVAs were used to analyze the effect of development temperature, sex on development time of all immature stages and the pupal mass for each sampling localities. Other ANOVA models were also fitted to analyze the effect of rearing temperature, sex and larval developmental time on the first and second principal component initially performed for each sampling localities. Growth rate was computed by dividing the natural logarithm of the pupal weight by the larval development time (see Gotthard *et al*., 1994).

All the thirteen wing-pattern measurements were reduced using a principal component analysis pooling data per sampling localities across sex and rearing temperature. The two or three-way ANOVA were used to analyze the effect of development temperature, sampling localities and sex on both PC1 and 2. Models were fitted including temperature, sampling localities and sex and their interactions as fixed factors. *Post-hoc* Tukey-Kramer Honestly Significant Difference (HSD) were used to compare means between specific levels of the factors where the ANOVA F-test was significant ($P < 0.05$).

The relationship between developmental time and PC1 was explored by performing piecewise linear regressions, with development time as dependent variable, using the 'segmented' package in R (Muggeo, 2008). The existence of one or several inflection points and significant differences in slopes was tested using Davies' tests (Muggeo, 2003), after which the positions of the inflection points (i.e. the developmental thresholds) and 95% confidence intervals were estimated.

II.3.5.4. Thermal tolerance in *Bicyclus dorothea*

The effects of habitat, sex and sampling sites on the critical thermal minimum and maximum, and the thermal tolerance breath were analyzed using a nested ANOVA model, with sampling sites nested in habitat. In order to meet ANOVA requirements, data were log-transformed to fit the normal distribution. Pair-wise comparisons were performed employing Tukey's HSD and all means were expressed as ±Standard Error (±SE). All statistical computations were done using the software R (Team, 2017) and all graphs were done using the package ggplot2 (Wickham, 2009).

CHAPTER III:

RESULTS AND DISCUSSION

III.1. Results

III.1.1. Bio-ecological aspects of *Bicyclus dorothea*

III.1.1.1. Morphological description of immature stages

Observations made during laboratory experiments showed that *Bicyclus dorothea* develops into immature and adult stages which are completely different regarding their morphology as in the case of the majority of holometabolous insects. Immature stages in *B. dorothea* are composed by the embryo (egg), larva and chrysalid (though a pre-chrysalid satge always precedes the chrysalid in the members of the genus *Bicyclus*). Four molting events were recorded during larval development which corresponded to five larval stages namely L_1, L_2, L_3, L_4 and L_5. Adult stage is represented by the emerged imago.

III.1.1.1.1. Morphological description of eggs

Bicyclus dorothea's eggs are white in color like those of other *Bicyclus* species. They are spherical with a diameter varying from 1 to 1.3 mm. The surface is not perfectly smooth; under the microscope, juxtaposed geometric shapes can be observed (Figure 13a). One day before eclosion, the upper pole of the eggs turns black (Figure 13b) representing the dark head capsule of the developing larva.

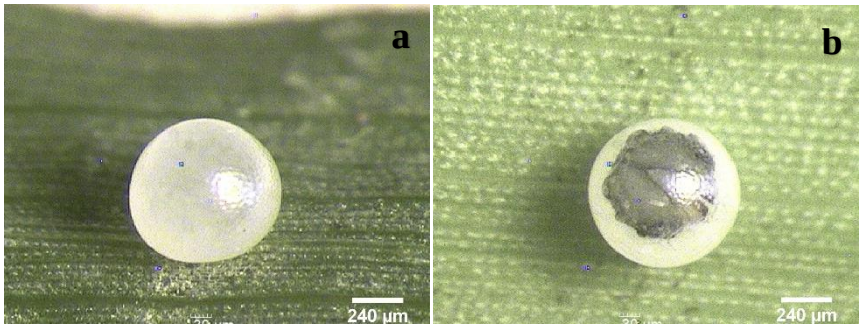

Figure 13. Eggs of *B. dorothea*: (a) newly laid egg; (b) egg on a lawn leaf with the head of the first instar caterpillar visible through the eggshell (Photo by Dongmo).

III.1.1.1.2. Morphological description of larvae

Bicyclus dorothea develops into five larval instars corresponding to four molting events. The newly emerged first instar larva is about 3 to 3.5 mm long, cylindrical white body covered by dorso-lateral rows of setae (Figure 14a). At the posterior end of the body, a pair of backward-pointing setae

can be easily observed. The head is dark and bears setae, protuberances, and one pair of short horns. First instar larvae are mobile immediately after eclosion and their body color turns greenish over time because of grass feeding.

The rupture of the head capsule indicates molting from one larval instar to the next. The second instar larva of *B. dorothea* is characterized by a brownish head capsule with a well distinguished pair of horns; the body is greenish and measures 4 to 6 mm long with more pronounced setae relative to the first instar (Figure 14b).

The following instars (third, fourth and fifth) differ from the previous two instars essentially in terms of body length and head capsule colors (Figure 14c-e). The body color is quite uniform and body length increases as the larvae grow. The third instar has a dark cephalic capsule with green striations in the front of the capsule and with distinguishable horns. The third instar stage takes about four to six days to complete. The next two molts bring the caterpillar to fourth and fifth instars and their average development time is respectively five and four days.

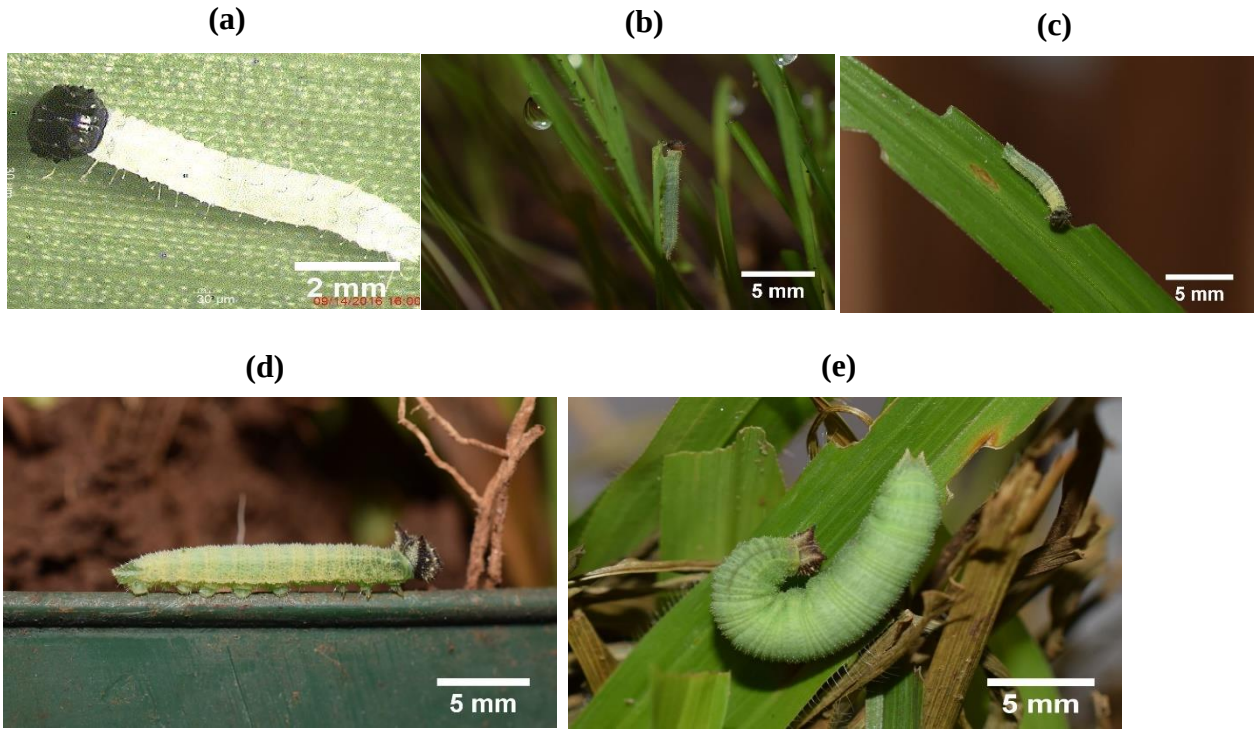

Figure 14. Pictures of *B. dorothea* larvae: (a) first instar larva; (b) second instar larva; (c) third instar larva; (d) fourth instar larva; (e) fifth instar larva (Photo by Dongmo).

III.1.1.1.3. Morphological description of pre-pupa and pupa

At the pre-pupal stage, the body of the caterpillar gradually shrinks in length and the caterpillar finds a spot on the underside of a leaf blade where it spins a silk pad (Figure 15a). It then brings its head towards the anal end to form a pre-pupa. This stage lasts one day at ambient temperature but can reach up to three days when reared at temperatures under 20°C.

The chrysalid of *B. dorothea* is green in color and approximately cylindrical in shape (Figure 15b). Pupation takes place as follows: once the pre-pupa is mature, a slot opens on the head capsule and the hanging caterpillar makes uncoordinated movements to facilitate shedding of the body cuticle toward the anal end. In most cases, the shed cuticle is removed from the fresh soft pupa which hardens few hours later. This stage lasts six to eight days under room temperature, and the pupa darkens in coloration one day before the imago emergence (Figure 15c).

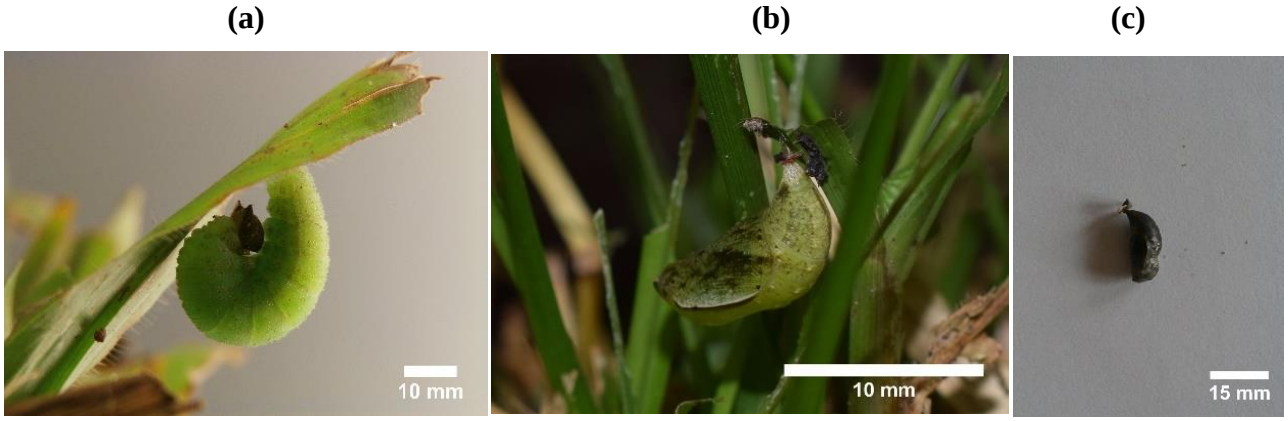

Figure 15. Pupal stages of *B. dorothea*: (a) pre-pupa; (b) early pupa; (c) pupa a few hours before the emergence of the adult (Photo by Dongmo).

III.1.1.1.4. Morphological description of adults

The morphological appearance of *B. dorothea* are displayed in Figures 16 a-d. Males differ from females by the size (males are smaller than females), the presence of differentiated hindwing scales called androconies which are organs responsible of the secretion of pheromones in males *Bicyclus*, and the color of the wings which is completely grey in females but in males, there is a dark zone on the costal and marginal areas of the fore and hindwings respectively (Figure 16 a-b).

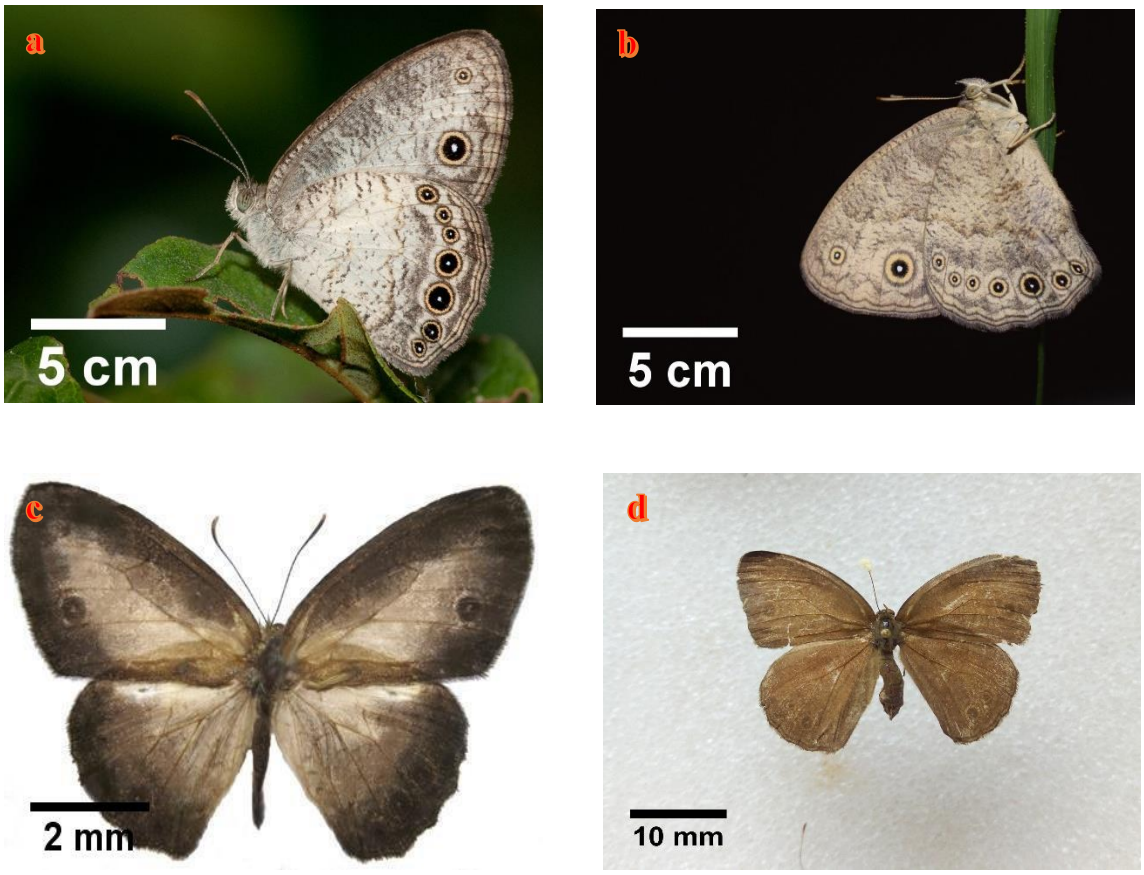

Figure 16. Morphology of adult *B. dorothea*: (a) ventral view of an adult male (Photo by Oskar Brattström); (b) ventral view of an adult female; (c) dorsal view of a male; (d) dorsal view of a female (Photo by Dongmo).

Morphological measurements of *B. dorothea* are presented in table I. Except the diameter of the seventh hindwing spot, all other morphological characters measured showed a significant difference between males and females. Generally, regarding the body size, female is larger than male, and the same trend is observed for the diameter of their wing spots (wing spots in females are larger than those of the males).

Table I. Morphological characteristics of a wild population *B. dorothea* collected in Mbalmayo. Values in brackets are the minimum and maximum values for each trait.

Morphological characteristics	Female	Male	$t_{(1, 137\ df)}$ value	*P-value*
Body length (head + thorax + abdomen)	13.65±0.17a (11.42-16.47)	15.68±0.08b (14.09-17.37)	13.831	<0.001
Length of the forewing	21.25±0.12a (19.34-23.32)	19.74±0.08b (18.25-21.31)	11.267	<0.001
Length of the hindwing	17.15±0.14a (14.44-18.81)	15.05±0.09b (11.72-17.19)	15.122	<0.001
Width of the forewing	13.24±0.09a (12.02-14.42)	11.72±0.06b (10.57-13.42)	7.917	<0.001
Width of the hindwing	14.68±0.30a (12.40-25.29)	12.77±0.09b (11.00-14.70)	15.381	<0.001
Diameter 1st eyespot forewing	1.63±0.51a (0.95-2.38)	1.42±0.03b (0.60-2.02)	5.063	<0.001
Diameter 2nd eyespot forewing	3.21±0.09a (1.31-4.29)	2.96±0.05b (1.90-3.80)	3.878	<0.001
Diameter 1st eyespot hind wing	1.33±0.03a (0.95-1.55)	1.18±0.03b (0.60-1.90)	4.630	<0.001
Diameter 2nd eyespot hind wing	1.18±0.04a (0.60-1.30)	1.08±0.02b (0.60-1.54)	2.760	0.006
Diameter 3rd eyespot hind wing	1.24±0.05a (0.48-1.43)	1.07±0.02b (0.60-1.67)	4.513	<0.001
Diameter 4th eyespot hind wing	2.10±0.06a (1.19-2.47)	1.81±0.03b (1.19-2.73)	5.811	<0.001
Diameter 5th eyespot hind wing	2.52±0.05a (1.90-3.21)	2.31±0.03b (1.55-2.98)	6.502	<0.001
Diameter 6th eyespot hind wing	1.64±0.05a (1.19-2.90)	1.48±0.02b (1.07-3.02)	3.902	<0.001
Diameter 7th eyespot hind wing	1.08±0.04a (0.48-1.54)	1.02±0.02a (0.71-1.43)	1.551	0.123

A student's t-test was applied to each character with sex as independent variable. Mean values with different letters in a row are significantly different at $P < 0.05$

III.1.1.2. Life history traits

Table II below gives the basic statistics of life history data such as development time (days ± SE) of each immature stage, the mean pupal mass (mg ± SE) and some reproductive parameters of females in *B. dorothea* rear under controlled laboratory conditions (25ºC, 75%RH and 12D:12L). In general, the development time of immature stages of males and females is almost the same. The pupal weight was greater in females than males, and adult females last twenty days more than males.

Table II. Mean developmental time (days ± SE) of immature stages, and some reproductive parameters of *B. dorothea* reared at a constant temperature of 25ºC in the laboratory

Development stages	Sex		*t- value*	*P-value*
	Male	**Female**		
Eggs	5.65 ± 0.16a	5.79 ± 0.07a	0.600	0.550
Larva	27.58 ± 0.51a	27.64 ± 0.46a	0.086	0.931
Prepupa	1.00 ± 0.00a	1.00 ± 0.00a	-	-
Pupa	9.92 ± 0.38a	9.24 ± 0.30a	-1.406	0.165
Eggs – pupa	43.15 ± 0.62a	42.67 ± 0.47a	-0.625	0.534
Eggs - adults	72.19 ± 2.29a	92.7 ± 1.32b	7.750	**<.0001**
Adults' survival	29.04 ± 2.12b	50.03 ± 1.27c	7.452	**<.0001**
Mean pupal mass	127.65 ± 1.66c	148.87 ± 1.50d	13.436	**<.0001**
Gross reproductive rate		48.04 ± 3.75		
Ro		12.80 ± 2.08		
Intrinsic rate of increase		0.035 ± 0.002		
Generation time		72.75 ± 0.83		
Lambda		1.03 ± 0.00		

A student's t-test was applied to each character with sex as independent variable. Mean values with different letters in a row are significantly different at $P < 0.05$

III.1.1.3. Geographic distribution and habitat preference of *B. dorothea* in Cameroon

Bicyclus dorothea is present in all the six regions but not in all the sites surveyed (Figure 17a). In general, *B. dorothea* distribution is restricted to low and mid-altitude habitats. Out of the 36 localities surveyed, only one individual was caught above 1000 m a.s.l (1032 m on Mont Cameroon). The field observations show that *B. dorothea* is rarely found in dense forest understory habitats, though occasionally a female will fly away from a threat into the understory. Generally, *B. dorothea* is found in degraded forests where colonies establish in clearings and road tracks receiving sun and where larval host plants and adult food resources are abundant (Figure 17b). Agricultural lands harboring canopy structures like palm farms, fruit trees and cacao farms are also good habitats for the species, probably because these microhabitats provide abundant food resources such as the decaying fruits on which they feed and probably due to less threats from natural enemies.

Flight activity starts as soon as the sun has risen, but under intense sunshine conditions adults will retreat into shade. Males are abundant throughout the day while female flight activity increases in the afternoon (from 14:00 to 17:00) when they display mating and oviposition behavior. *Bicyclus dorothea* individuals usually fly alone, although it is common to see contest behaviors in males.

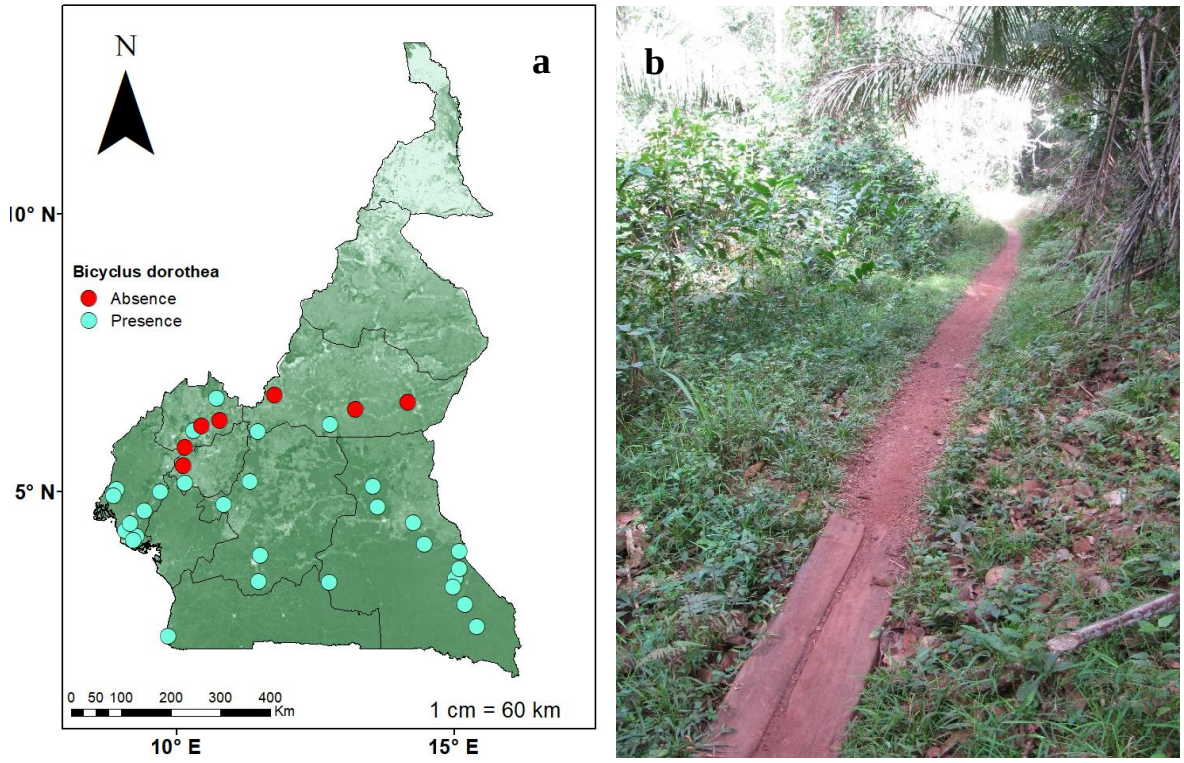

Figure 17. Occurrence of *B. dorothea* in Cameroon and its microhabitat. a) Map showing the presence and absence of *B. dorothea* after a survey in Cameroon; b) suitable habitat for *B. dorothea* in the Dja wildlife reserve (Photo and map by Dongmo).

III.1.2. Seasonal polyphenism in wing pattern of *Bicyclus dorothea* in the wild

A total of 1498 butterflies were captured for two years (2015-2016) of sampling in four localities representing two distinct habitats. More males than females were caught at each site suggesting a biased sex ratio in wild populations of *B. dorothea* (Table III). The principal component analysis performed on the morphological data recorded 80.3% of the total variation of the first two principal components (PC1 and PC2) in population from Ako, 73.39% in Ndikiniméki, 75.01% in Mbalmayo and 71.92% in Somalomo. Eigenvalues were obtained from morphological data of males and females pooled together. The diameter of wing spots loaded heavily on PC1 while the length and the width of the fore and hindwings had the highest loadings on PC2 in all populations (Table IV).

PC1 and PC2 varied across seasons and habitat (Figures 18 and 19), but also across seasons and sampling localities (Figures 18 and 19).

Table III. Number of butterflies captured during two years at each sampling sites

Sampling sites	Habitat	Sex	Seasons				Total
			Wet 2015	Dry 2015	Wet 2016	Dry 2016	
Ako	Ecotone	Males	83	69	44	38	369
		Females	31	26	50	28	
Ndikiniméki		Males	72	69	54	43	382
		Females	50	27	39	28	
Mbalmayo	Forest	Males	74	69	67	58	434
		Females	54	34	56	22	
Somalomo		Males	30	93	36	28	313
		Females	34	36	35	21	

The first principal component represents the main wing pattern characters varying across season, habitat and sampling localities (Figure 18), while PC2 showed less variation when considering these factors. This result suggests that the length and the width of the wings are not (or are slightly) seasonally plastic and are less affected by habitat or sampling localities. Within each habitat, PC1 fluctuated seasonally and simultaneously, but the pattern of variation was not identical. In order to give an illustration of how the diameter of the eyespots varied within seasons, habitat and sampling localities, the diameters of the second ocelli of the forewing and the fifth of the hindwing were plotted. Figure 21 (page 58) shows that the diameter of ocelli varied substantially from one season to another in ecotone populations (Ako and Ndikiniméki) but varied slightly in the forest populations (Figure 21).

Table IV. Component weights and eigenvalues for the principal component analysis with data of all localities and seasons pooled together in males and females. Eigenvalues are expressed as percentage of total variance accounted for by the first and second principal components.

	PC1 wing spot				PC2 body size			
Morphological parameters	Ako	Ndikiniméki	Mbalmayo	Somalomo	Ako	Ndikiniméki	Mbalmayo	Somalomo
Length of the forewing	0.156	0.168	0.234	0.193	0.557	0.446	-0.444	0.544
Length of the hind wing	0.166	0.209	0.223	0.212	0.546	0.447	-0.456	0.541
Width of the forewing	-0.026	0.212	0.218	-0.014	0.009	0.439	-0.409	0.021
Width of the hind wing	0.164	0.230	0.233	0.219	0.557	0.473	-0.459	0.522
Diameter 1st eyespot forewing	0.319	0.318	0.302	0.328	-0.103	-0.174	0.161	-0.107
Diameter 2nd eyespot forewing	0.326	0.326	0.308	0.331	-0.124	-0.155	0.157	-0.121
Diameter 1st eyespot hind wing	0.310	0.288	0.261	0.280	-0.0003	-0.034	0.096	-0.102
Diameter 2nd eyespot hind wing	0.326	0.320	0.301	0.321	-0.083	-0.174	0.230	-0.182
Diameter 3rd eyespot hind wing	0.328	0.333	0.323	0.333	-0.083	-0.139	0.172	-0.136
Diameter 4th eyespot hind wing	0.329	0.334	0.329	0.337	-0.117	-0.151	0.129	-0.112
Diameter 5th eyespot hind wing	0.328	0.325	0.331	0.332	-0.151	-0.191	0.155	-0.140
Diameter 6th eyespot hind wing	0.322	0.300	0.295	0.320	-0.110	-0.132	0.143	-0.122
Diameter 7th eyespot hind wing	0.282	0.125	0.189	0.177	-0.055	-0.007	0.099	-0.011
Eigenvalue	63.09%	57.18%	57.25%	57.08%	17.18%	16.20%	17.75%	14.84%

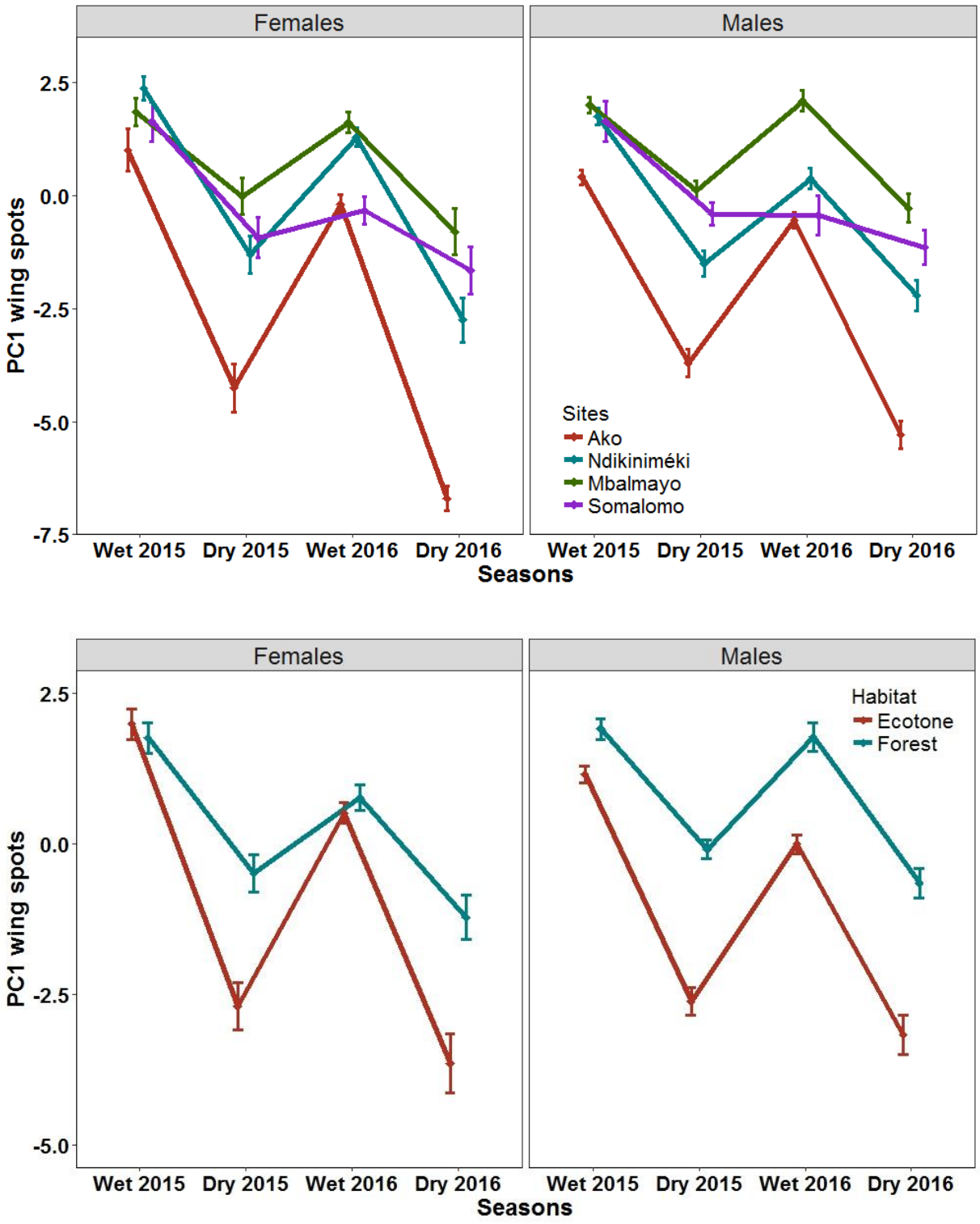

Figure 18. Seasonal changes in the first principal component in *B. dorothea*'s wing patterns in different populations in Cameroon inhabiting forest and ecotone habitats over the transition from a wet to a dry season.

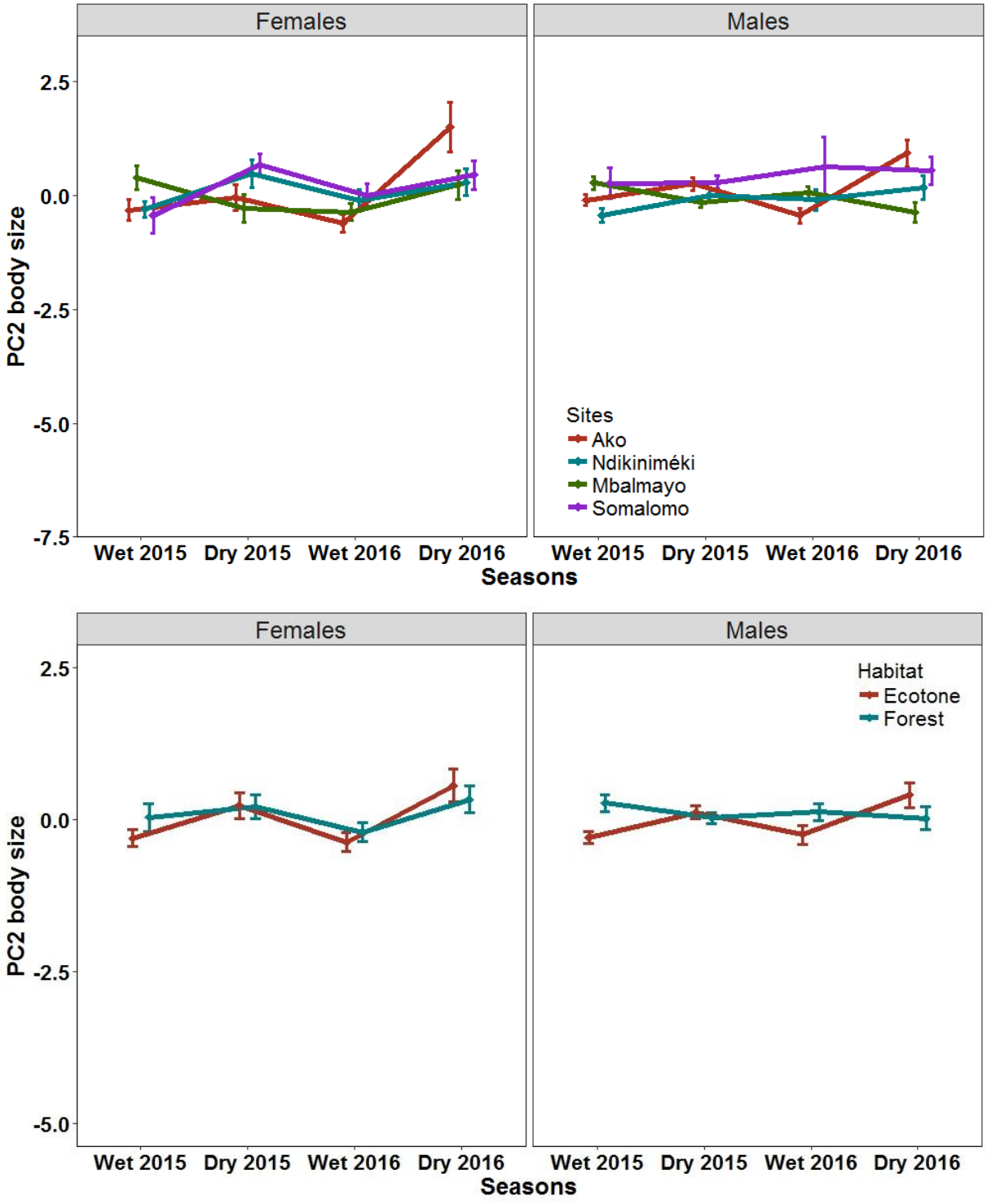

Figure 19. Seasonal changes in the first principal component in *B. dorothea*'s wing patterns in different populations in Cameroon inhabiting forest and ecotone habitats over the transition from a wet to a dry season.

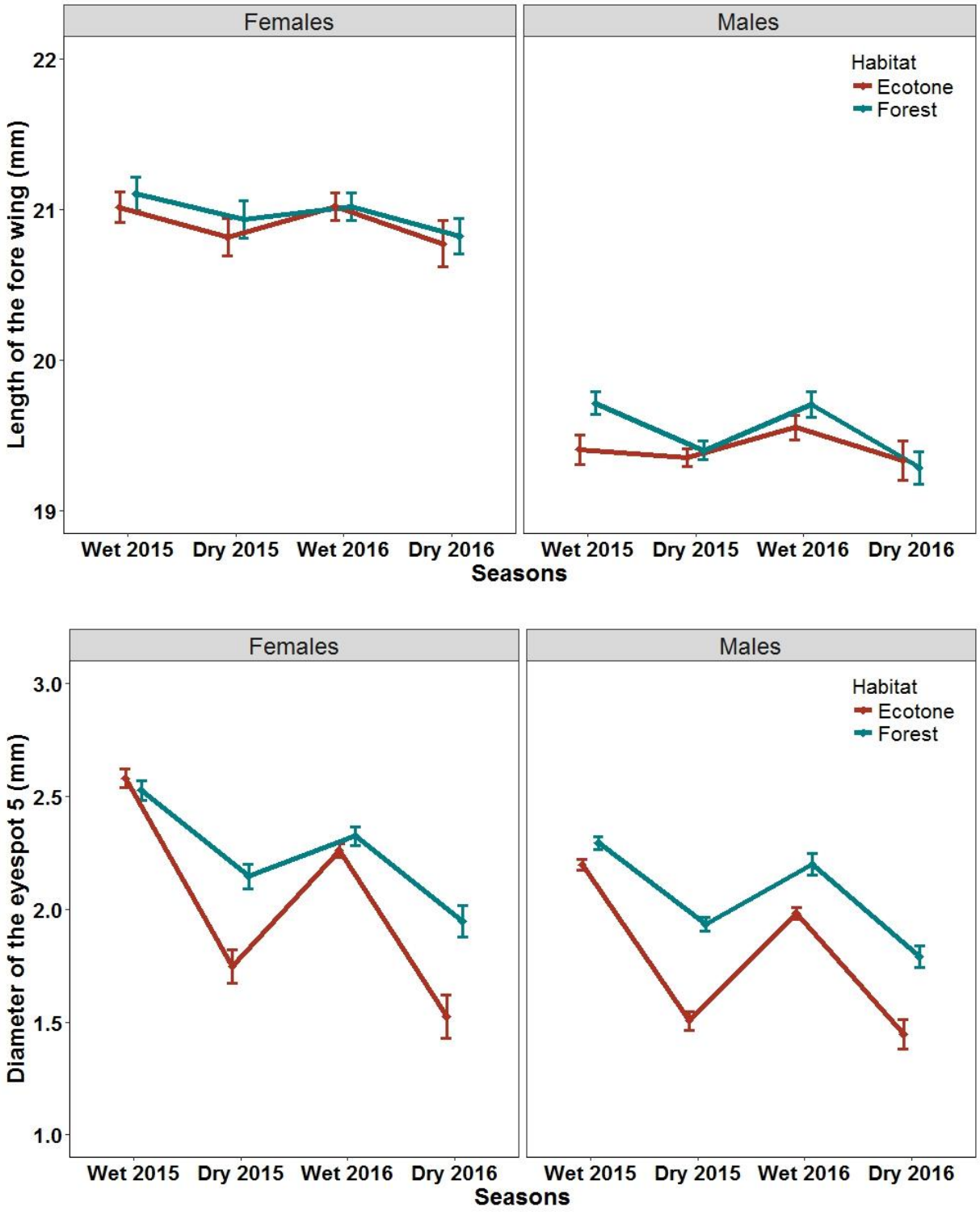

Figure 20. Seasonal changes in the length of the forewing and the second diameter of the forewing of *B. dorothea* wings in different populations in Cameroon inhabiting forest and ecotone habitats over the transition of the wet to dry seasons.

Results of the nested analysis of variance (ANOVA) performed using PC1 and PC2 show a large response for PC1 to season, sampling localities but not to sex. The interaction between seasons and sex, seasons and localities were also significant. There was a significant response of PC2 to season, sampling localities and the interaction between seasons and localities; all other factors show no significant effect to PC2 (Table V).

Table V. Statistical results of the nested ANOVA models for the relationships between the principal component 1 and 2 and environmental factors

Response trait	**Factors**	***df***	**Mean sq.**	***F*- value**	**p-value**
PC1	Sex	1	0	0.003	0.960
	Seasons	3	847.0	214.875	**<0.001**
	Localities	3	473.9	120.233	**<0.001**
	Seasons*Localities	9	49.2	12.471	**<0.001**
	Seasons*Sex	3	10.8	2.728	**0.043**
	Sex*Localities	3	8.0	2.024	0.108
	Seasons*Sex*Localities	9	2.4	0.610	0.789
PC2	Sex	1	0.460	0.241	0.623
	Seasons	3	11.828	6.198	**0.000**
	Localities	3	5.919	3.101	**0.026**
	Seasons*Localities	9	9.073	4.754	**<.001**
	Seasons*Sex	3	2.709	1.420	0.235
	Sex*Localities	3	1.782	0.934	0.423
	Seasons*Sex*Localities	9	2.143	1.123	0.342

P values in bold are significantly different at 0.05

The analysis of each trait using the multivariate ANOVAs with sampling localities, seasons and sex as fixed factors shows a low response for the length of the fore and hind wings, the width of the fore wings while these traits did not show any significant differences when fixed factors were combined in the model, except the width of the hind wing (Table VI). The diameter of all eyespots differs significantly between the sampling localities, seasons and sex and their interaction while the interaction between sampling localities and sex was not significant for the diameter of the second, third, sixth and seventh eyespot of the hind wing. Except for the seventh eyespot of the hind wing, the interaction between the sampling localities, season and sex had no significant effect on the diameter of other eyespot of both fore and hind wing.

Table VI. F-ratios obtained from multifactor analysis of variance MANOVA for the indicated characters and factors. Significance level <0.001, ns = not significant

Traits	Local	Season	Sex	Local*Seas	Local*Sex	Seas*Sex	Local*Seas*Sex
LFW	3.30	5.32	809.63	2.71	0.61 (ns)	0.40 (ns)	0.44 (ns)
LHW	8.21	4.21	2035.22	1.19 (ns)	3.05	0.50 (ns)	1.65 (ns)
WFW	36.21	44.66	2115.02	2.83 (ns)	3.45 (ns)	3.41 (ns)	3.81 (ns)
WHW	0.80 (ns	1.04 (ns)	0.14 (ns)	0.55 (ns)	0.31 (ns)	0.48 (ns)	0.20 (ns)
Diameter of the eyespot fore wing							
D1	78.00	195.20	192.08	11.59	0.77 (ns)	3.40	0.81 (ns)
D2	78.00	279.07	176.57	15.50	0.88 (ns)	3.40	1.10 (ns)
Diameter of the eyespot hind wing							
D1	88.57	63.93	257.12	9.32	7.25	5.02	0.73 (ns)
D2	77.35	146.63	127.40	29.84	3.00	1.68 (ns)	0.42 (ns)
D3	68.60	156.46	223.11	10.56	1.30 (ns)	2.50 (ns)	0.50 (ns)
D4	51.18	223.70	260.70	15.04	1.24	2.40 (ns)	0.93 (ns)
D5	101.43	226.95	149.78	16.02	0.48 (ns)	1.10 (ns)	0.78 (ns)
D6	130.47	118.05	118.70	1.20 (ns)	0.30 (ns)	4.62	0.77 (ns)
D7	42.90	23.20	25.04	4.62	0.25 (ns)	1.20 (ns)	0.78

Local = Localities, Seas = Season, LFW = length of the fore wing, LHW = length of the hind wing, WFW = width of the fore wing, WHW = width of the hind wing, D1 = diameter of the first eyespot, D2 = diameter of the second eyespot, D3 = diameter of the third eyespot, D4 = diameter of the fourth eyespot, D5 = diameter of the fifth eyespot, D6 = diameter of the sixth eyespot and D7 = diameter of the seventh eyespot.

Table VII gives the rate of change of each trait calculated for each habitat during the transition between the wet and the dry season. This table shows a relatively lower change in the length and width of the fore and hind wings and the length of the body. On the other hand, plastic change in the diameter of each eyespot over the wet and dry season was substantial for both Ako and Ndikiniméki (ecotone) populations compared with their forest counterparts (Mbalmayo and Somalomo). The change eyespots diameters of ecotone populations were approximatively 2-fold as much as those of the forest populations. Moreover, among ecotone population individuals from Ako tend to be more plastic when comparing the rate of change of each of their eyespot with those of individuals originated from Ndikiniméki. The rate of change of the diameter of the eyespot in both males and females for Mbalmayo and Somalomo populations was quite similar.

Table VII. Amplitude in % (difference of the wet and dry season forms the average phenotype) in *Bicyclus dorothea* sampled at four different localities of Cameroon.

Traits	Sampling localities							
	Ako		Ndikiniméki		Mbalmayo		Somalomo	
	Male	Female	Male	Female	Male	Female	Male	Female
LB	3.19	4.88	1.60	0.79	3.82	0.20	4.39	3.92
LFW	1.46	2.12	0.52	0.60	2.08	1.38	0.66	-0.83
LHW	1.52	3.90	-0.22	1.83	1.62	0.74	1.37	-2.16
WFW	3.21	4.68	2.17	3.21	4.75	3.46	8.12	2.38
WHW	2.14	4.19	1.65	2.31	3.35	1.95	1.87	-1.28
Diameter eyespot fore wing								
D_1	38.09	42.04	34.72	42.67	19.24	19.47	13.37	19.01
D_2	45.47	45.01	34.25	39.88	19.96	18.64	21.26	25.07
Diameter eyespot hind wing								
D_1	33.80	29.97	21.33	25.82	4.85	13.32	14.98	17.60
D_2	38.04	35.36	25.90	33.50	15.83	17.37	14.78	22.80
D_3	39.63	38.86	27.82	34.31	15.34	15.83	17.24	21.78
D_4	38.93	42.00	29.94	36.20	18.46	18.15	13.71	19.81
D_5	37.17	37.95	26.59	30.29	17.36	15.71	13.01	17.92
D_6	30.36	33.79	16.44	16.74	10.82	7.83	10.42	17.47
D_7	20.20	32.00	10.72	14.21	4.65	4.09	18.47	17.15

LB = Length of the body, LFW = length of the fore wing, LHW = length of the hind wing, WFW = width of the fore wing, WHW = width of the hind wing, D_1 = diameter of the first eyespot, D_2 = diameter of the second eyespot, D_3 = diameter of the third eyespot, D_4 = diameter of the fourth eyespot, D_5 = diameter of the fifth eyespot, D_6 = diameter of the sixth eyespot and D_7 = diameter of the seventh eyespot.

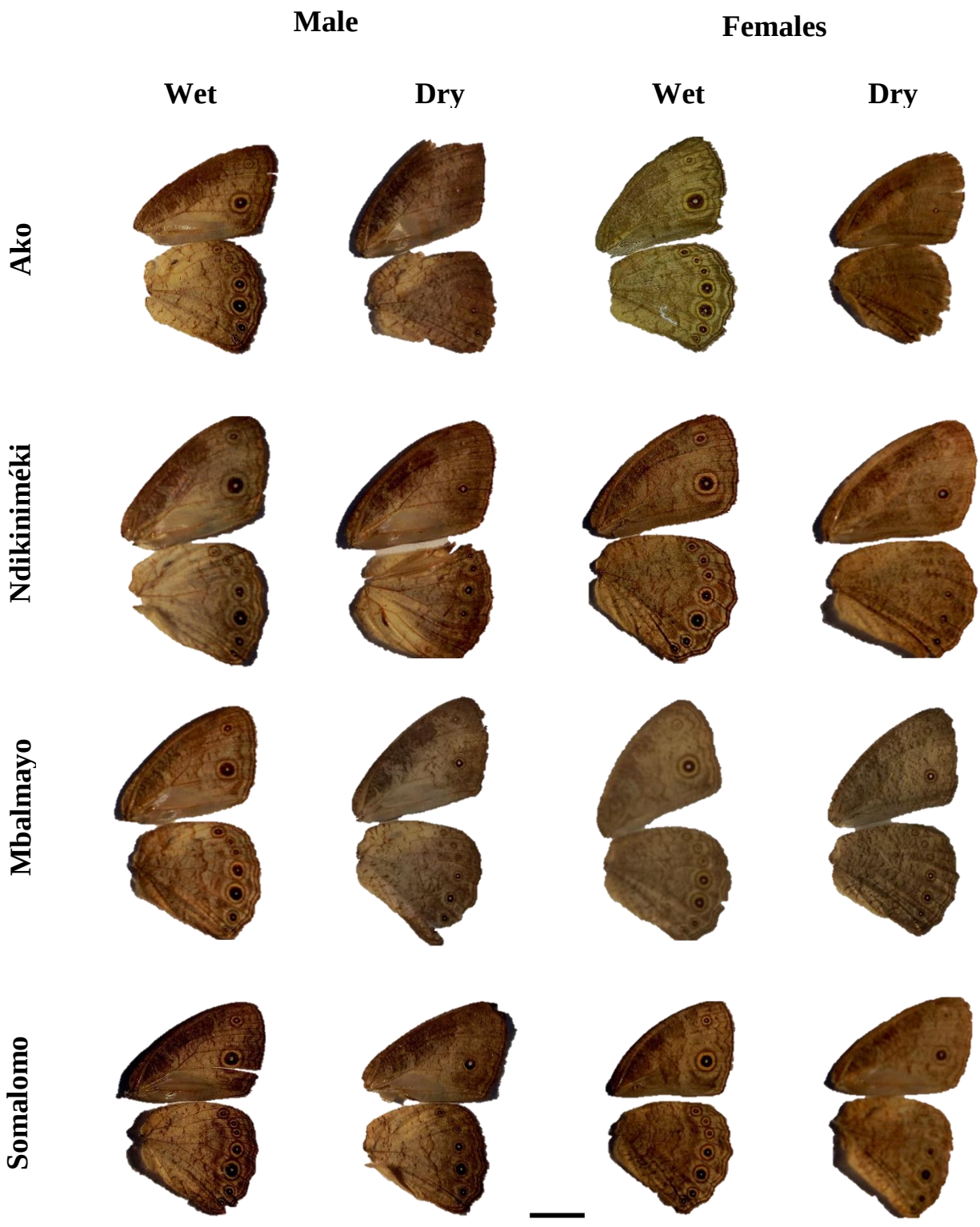

Figure 21. Wing pattern variation of *B. dorothea* individuals across seasons (wet and dry) and sampling localities of Cameroon. Scale bar: 5cm

III.1.3. Temperature-induced plasticity in different populations of *Bicyclus dorothea*

III.1.3.1. Life history traits

III.1.3.1.1. Development time of immature stages

Sampling localities ($F_{3, 128}$ = 1.49, p = 0.220) and habitat type ($F_{1, 128}$ = 0.259, p = 0.611) do not affect egg development time, while rearing temperature ($F_{2, 128}$ = 52,051, p = 0.0001) has a highly significant effect on this parameter. The development of eggs was long at low temperature for both habitats and sampling localities (22 > 26 > 30°C, see Figure 22). No significant interaction between habitat and temperature was observed ($F_{2, 128}$ = 1.284, p = 0.280), probably due to a relatively small difference between populations from both habitat at all rearing temperatures (Figure 22) while the interaction between sampling localities and temperature affected egg development ($F_{3, 128}$ = 2.80, p = 0.01).

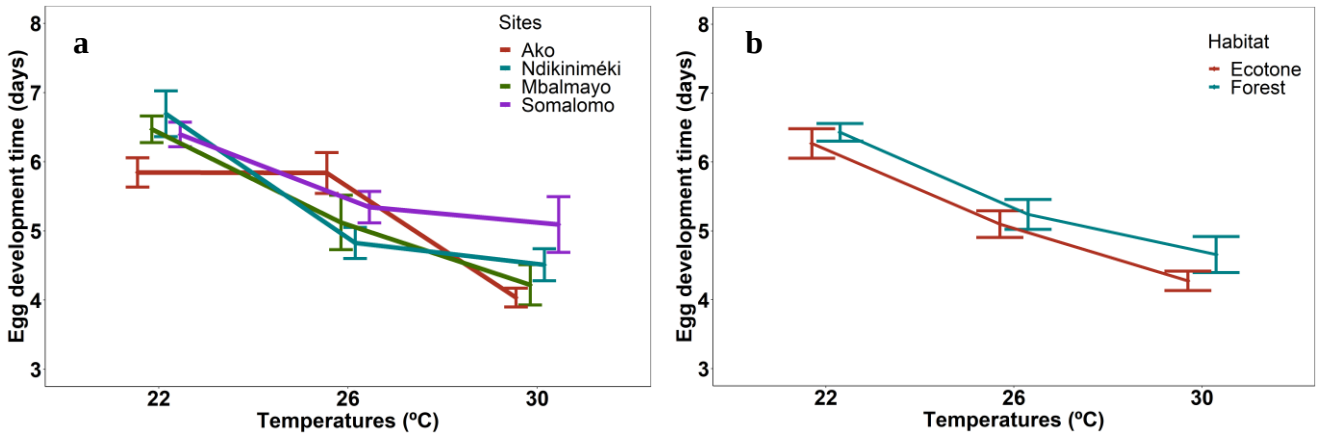

Figure 22. Effects of developmental temperature on the development time of eggs for: a) each sampling locality and b) habitat type.

The following factors of the ANOVA model, that is sampling localities ($F_{3, 128}$ = 3.577, p = 0.015), habitat ($F_{1, 128}$ = 4.784, p = 0.030), temperature ($F_{2, 128}$ = 145.177, p < 0.0001) and their interaction (sampling localities*Temperature ($F_{6,128}$ = 9.800, p = 7.16e-09) and Habitat*Temperature ($F_{6, 128}$ = 6.975, p = 0.001) had significant effect on larval development at each rearing temperature regime (average difference: 3.00% for forest and ecotone habitats) (Figure 23)

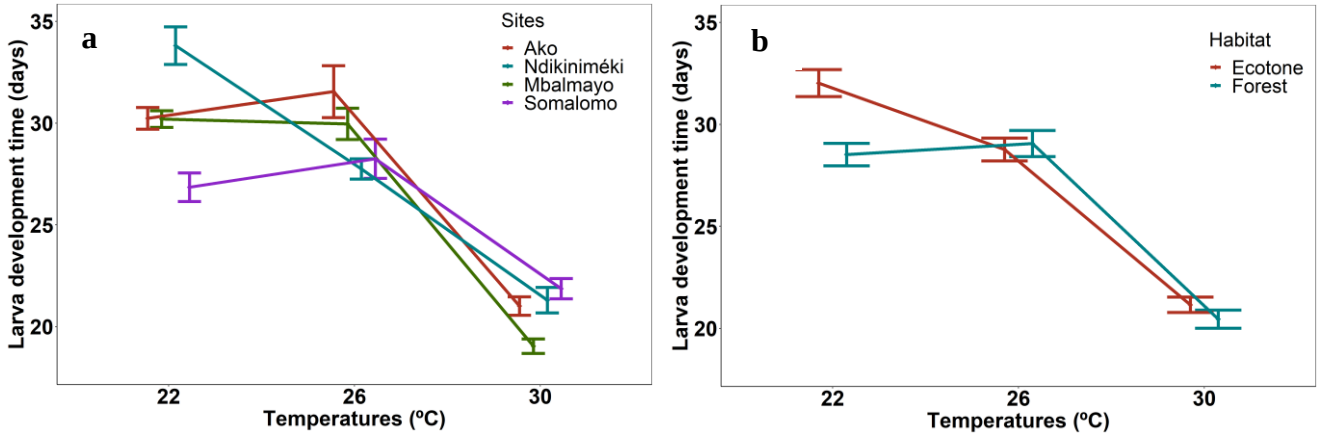

Figure 23. Effects of developmental temperature on the development time of larvae for: a) each sampling locality and b) habitat type.

Pupal development time was affected by rearing temperature in males ($F_{2, 128}$ = 40.493, p < 0.0001) and females ($F_{2, 128}$ = 48.349, p < 0.0001); at 22ºC, pupal development time was longer in both males and females compared with 26 and 30ºC in which the development time was the same (22 > 26 = 30ºC). Habitat type ($F_{1, 128}$ = 0.006, p = 0.940) and its interaction with temperature ($F_{2, 128}$ = 6.975, p = 0.637; $F_{2, 128}$ = 1.098, p = 0.337) did not have significant effect on males and females respectively (Figure 24). The same trend was observed for sampling localities where no effect was observed on pupal development time in males ($F_{2, 128}$ = 0.696, p < 0.935) and females ($F_{6, 128}$ = 0.141, p < 0.935). The interaction between sampling localities and rearing temperatures did not have a significant effect on pupal developmental time in males ($F_{6, 128}$ = 0.696, p = 0.654) and females ($F_{6, 128}$ = 0.696, p = 0.654).

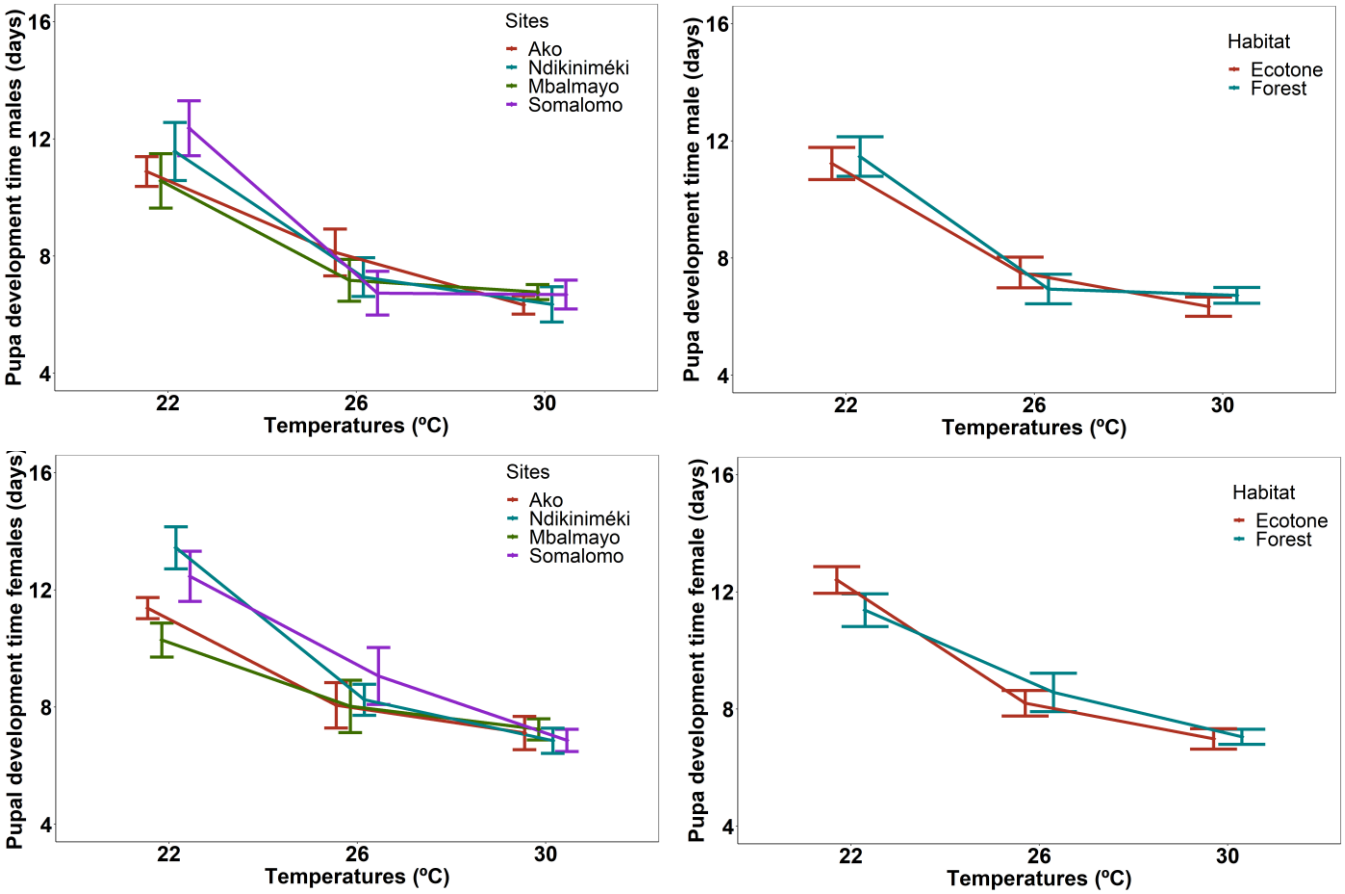

Figure 24. Effects of developmental temperature on pupal development time of males and females across sampling localities and habitat type.

Total development time (from egg to adults) was not affected by habitat in both males ($F_{1, 128}$ = 3.292, p = 0.072) and females ($F_{1, 128}$ = 3.292, p = 0.051) while the interaction between temperature and habitat had significant effect in males ($F_{2, 128}$ = 3.436, p = 0.035) and females ($F_{2, 128}$ = 12.413, p < 0.0001). Sampling localities did not affect total development time in males ($F_{3, 128}$ = 1.759, p = 0.158) or females ($F_{1, 128}$ = 3.292, p = 0.051) but its interaction with temperature was highly significant in both sexes (males: $F_{6, 128}$ = 88.9, p < 0.0001; females: $F_{6, 128}$ = 98.1, p < 0.0001) Figure 25 shows the reaction norms of total development time in response to temperature, habitat and sampling localities in both males and females.

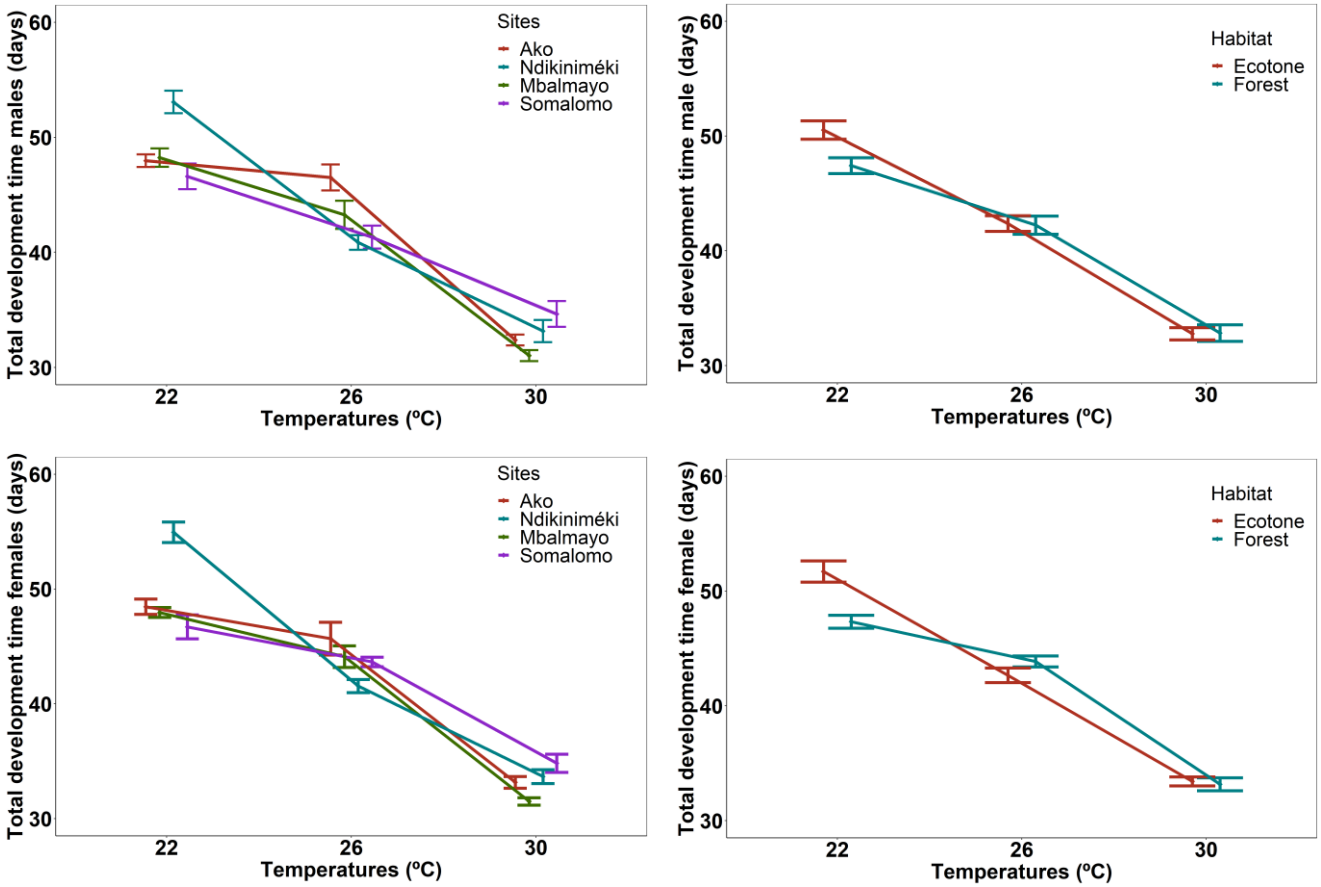

Figure 25. Effects of developmental temperature on total development time (egg to adult) of males and females across sampling localities and habitat type.

III.1.3.1.2. Pupal weight

There was a significant effect of habitat ($F_{1, 1558}$ = 448,475, p < 0.0001), temperature ($F_{2, 1558}$ = 94.405, p < 0.0001) and sex ($F_{1, 1558}$ = 1770.262, p < 0.0001) on pupal weight (Figure 26). There was no significant interaction between habitat and temperature ($F_{2, 1558}$ = 2.294, p = 0.101), habitat and sex ($F_{1, 1558}$ = 0.394, p = 0.530) while there was a significant interaction between temperature and sex ($F_{2, 1558}$ = 3.268, p = 0.038), temperature, habitat and sex ($F_{2, 1558}$ = 810, p = 0.007). Sampling localities show differences in pupal weight reared at all temperature regimes in males ($F_{3, 735}$ = 79.502, p < 0.0001) and females ($F_{3, 811}$ = 42.950, p < 0.0001). For both sexes, localities situated at forest habitat had heavier pupae than those from ecotone habitat at all temperatures (average difference = 4.39% and 3.37% for males and females respectively, Mbalmayo > Somalomo > Ndikiniméki = Ako) and moreover, per habitat and per sex, between-temperature differences were significant in *post hoc*

tests (22 > 26 > 30°C). Pupal weight was consistently lower in males than in females for both habitats (average difference = 10.97%).

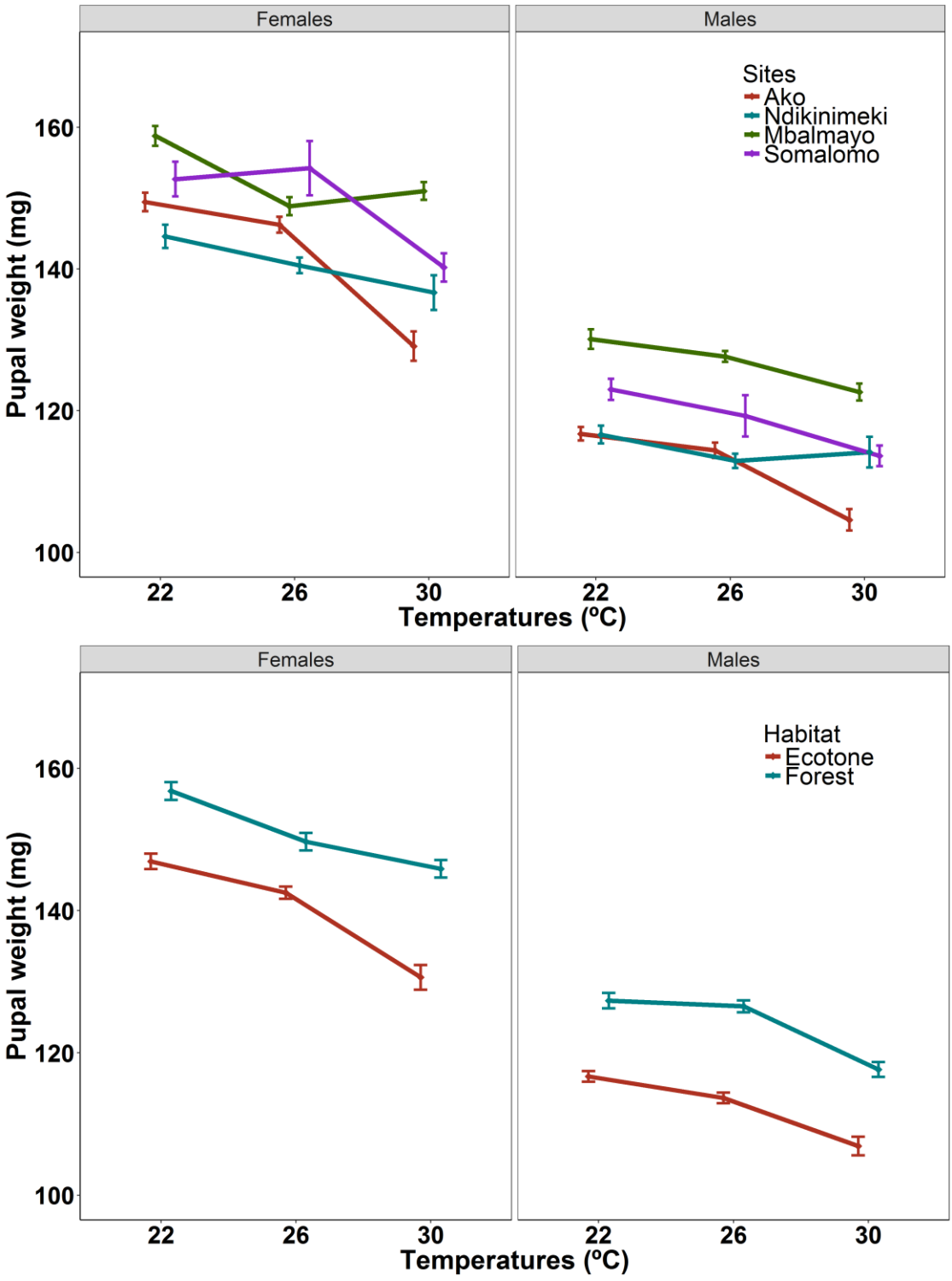

Figure 26. Effects of developmental temperature on pupal weight (males and females) across sampling localities and habitat type.

III.1.3.2. Wing pattern

In total, 1052 second-generation individuals from all sampling localities were reared in the temperature reaction norm experiment. The PCA performed shows significant interactions between different populations and temperature for wing pattern elements, indicating that populations respond differently to developmental temperature. The first two principal components (PC1 and PC2) of the 13 wing pattern measurements following the results of the principal component analysis are given in Table VIII. The total variation of the first two principal component combined (PC1 and PC2) was 87.90%, 85.76%, 78.64% and 85.36 for Ako, Ndikiniméki, Mbalmayo and Somalomo respectively. In all localities, the diameter of all the eyespots loaded heavily on the first principal component (PC1) while the length and the width of both fore and hind wings were captured by the second principal component (PC2) (Table VIII).

The variation of PCs with respect to rearing temperatures, sampling localities and habitat type are shown in Figure 26. In both males and females, populations from Ako and Ndikiniméki show a nearly linear or continuous relationship between the PC1 and rearing temperatures, while those from Mbalmayo and Somalomo show a discontinuous relationship (characterized by broken lines) for the same parameters (that is PC1 and rearing temperature). The reaction norm line shows that populations from ecotone habitat (Ako and Ndikiniméki) have larger eyespot at 30ºC in females than populations from forest habitat (Mbalmayo and Somalomo) while in males the eyespots were more pronounced at 22 and 26ºC in ecotone populations (Figure 27). The same observation was noted for the variation of the diameter of the second eyespot of the forewing (Figure 29). However, the relationship between the body size of adults decreases almost linearly with respect to rearing temperature in all populations with slight discontinuity in forest populations (Mbalmayo and Somalomo). Individuals reared at low temperature (22ºC) tended to be larger in size than those reared at high temperature (30ºC) in all populations and habitat (Figure 28).

Table VIII. Component weights and eigenvalues for the principal component analysis with data of all localities and rearing temperatures pooled together in males and females. Eigenvalues are expressed as percentage of total variance accounted for the first and the second principal components.

	PC1 wing spot				PC2 body size			
Morphological parameters	Ako	Ndikiniméki	Mbalmayo	Somalomo	Ako	Ndikiniméki	Mbalmayo	Somalomo
Length of the forewing	-0.073	0.124	-0.096	-0.134	0.495	-0.473	0.483	0.474
Length of the hind wing	-0.103	0.099	-0.092	-0.123	0.489	-0.497	0.494	0.489
Width of the forewing	-0.106	0.068	-0.070	-0.110	0.485	-0.505	0.500	0.493
Width of the hind wing	-0.108	0.050	-0.053	-0.067	0.467	-0.491	0.487	0.492
Diameter 1st eyespot forewing	-0.325	-0.328	0.326	0.312	-0.111	-0.042	0.041	0.060
Diameter 2nd eyespot forewing	-0.332	-0.343	0.340	0.340	-0.124	-0.034	0.021	0.050
Diameter 1st eyespot hind wing	-0.312	-0.311	0.305	0.306	-0.090	-0.029	0.006	0.112
Diameter 2nd eyespot hind wing	-0.333	-0.337	0.342	0.333	-0.048	-0.052	0.072	0.071
Diameter 3rd eyespot hind wing	-0.333	-0.342	0.336	0.331	-0.030	-0.058	0.038	0.072
Diameter 4th eyespot hind wing	-0.340	-0.343	0.338	0.343	-0.077	-0.079	0.046	0.060
Diameter 5th eyespot hind wing	-0.340	-0.331	0.359	0.343	-0.097	-0.075	0.051	0.057
Diameter 6th eyespot hind wing	-0.331	-0.330	0.339	0.333	-0.051	-0.078	0.081	0.085
Diameter 7th eyespot hind wing	-0.294	-0.275	0.270	0.273	-0.060	-0.064	0.115	0.088
Eigenvalue (%)	61.41	59.90	52.60	59.78	26.49	25.86	26.05	25.57

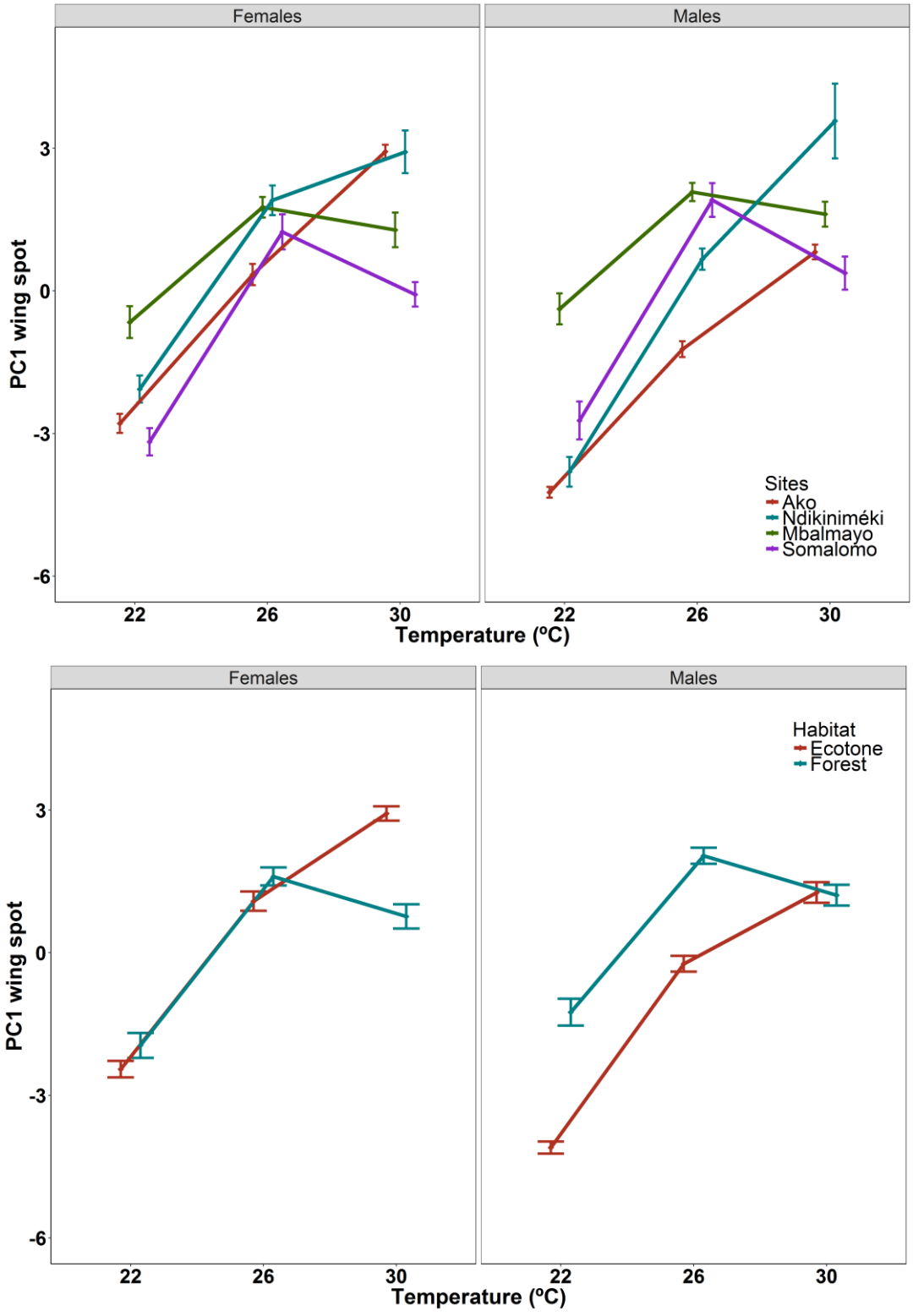

Figure 27. Effects of developmental temperature on the diameter of the wing spots in second-generation males and females' individuals of *B. dorothea*. Populations originated from four localities (Ako, Ndikiniméki, Mbalmayo and Somalomo) corresponding to two habitats (forest and ecotone).

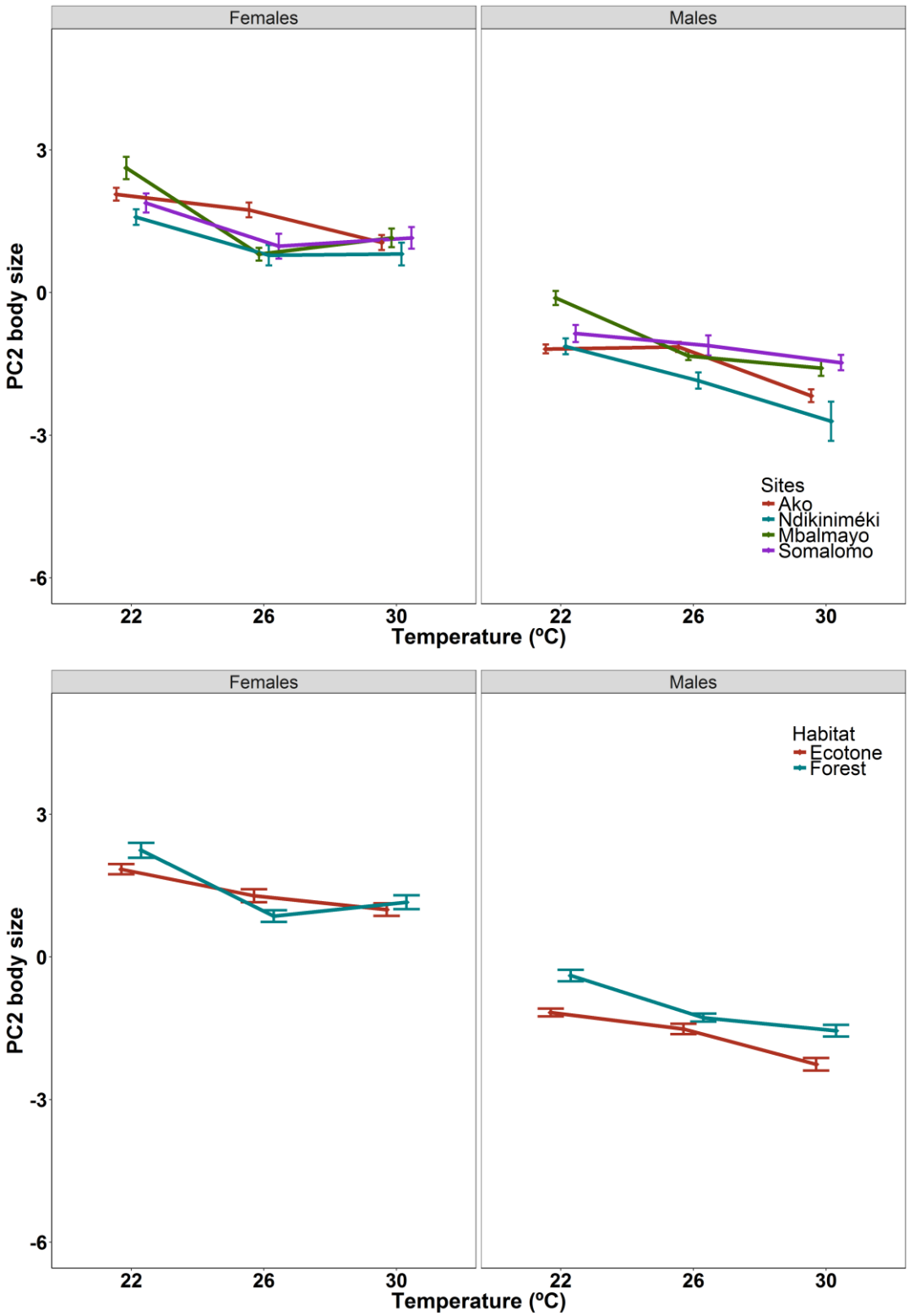

Figure 28. Effects of developmental temperature on the wing's size in second-generation males and females' individuals of *B. dorothea*. Populations originated from four localities (Ako, Ndikiniméki, Mbalmayo and Somalomo) corresponding to two habitats (forest and ecotone).

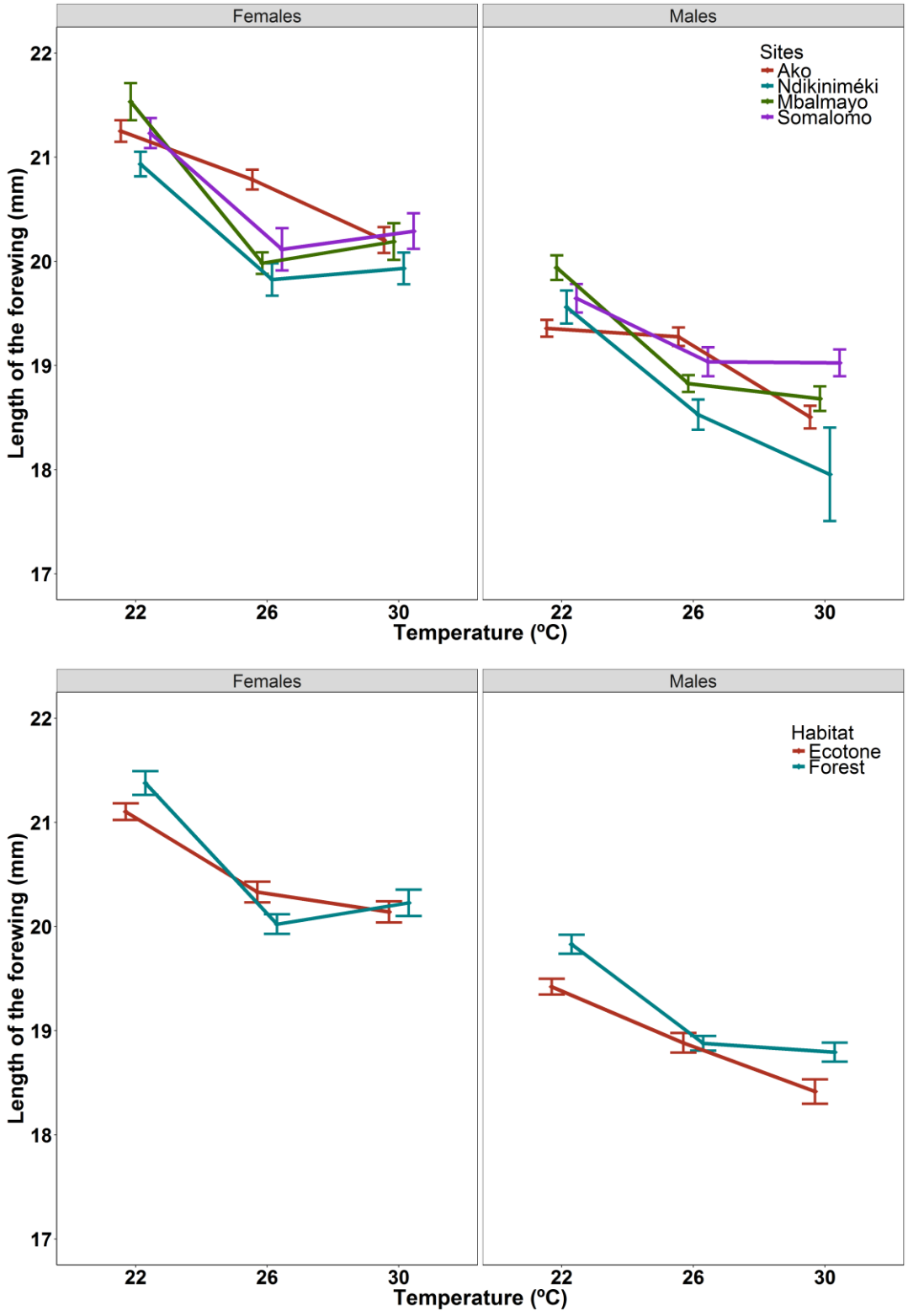

Figure 29. Effects of developmental temperature on the length of the forewing in second-generation males and females' individuals of *B. dorothea*. Populations originated from four localities (Ako, Ndikiniméki, Mbalmayo and Somalomo) corresponding to two habitat types (Forest and ecotone).

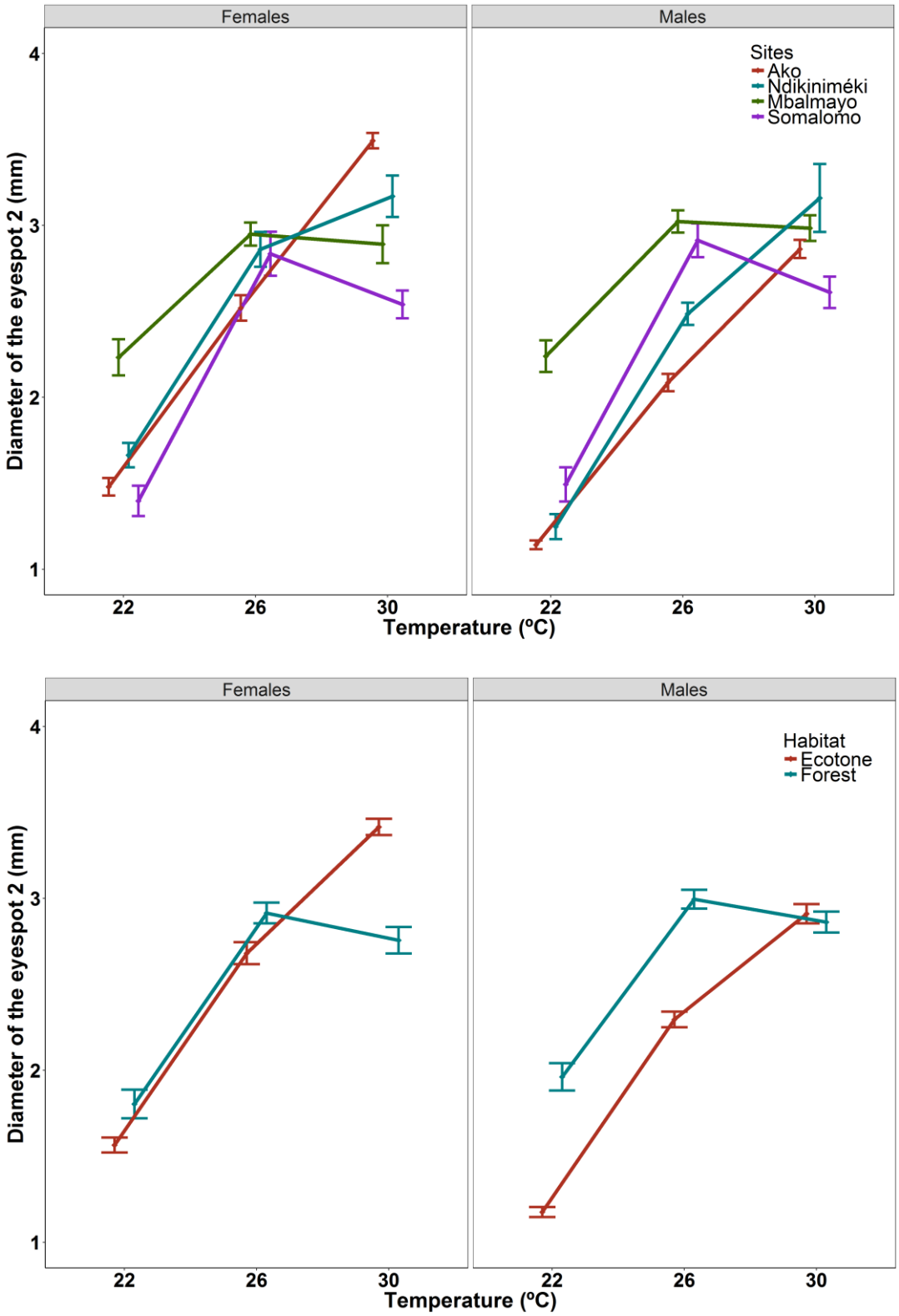

Figure 30. Effects of developmental temperature on the diameter of the second wing spot in second-generation males and females' individuals of *B. dorothea*. Populations originated from four localities (Ako, Ndikiniméki, Mbalmayo and Somalomo) corresponding to two habitat types (forest and ecotone).

The analysis of variance (ANOVA) performed using PC1 and PC2 show high response to rearing temperature, sampling localities and sex. Only the interaction between temperature, sex and temperature, sex and sampling localities was not significant for PC1 while for PC2, the interaction between temperature, sex and localities was not significant (Table IX). Subsequent *post hoc* testing showed a significant or no differences between sampling localities for each temperature for PC1 (Ako: 22 < 26 < 30°C, Ndikiniméki: 22 < 26 < 30°C; Mbalmayo: 22 < 26 = 30°C, Somalomo: 22 < 26 < 30°C) and the same trend was observed for PC2 (Ako: 22 = 26 > 30°C, Ndikiniméki: 22 > 26 = 30°C; Mbalmayo: 22 > 26 = 30°C, Somalomo: 22 > 26 = 30°C)

Table IX. Statistical results of the nested ANOVA models for the relationships between the principal component 1 and 2 and rearing temperatures

Response trait	Factors	*df*	Mean sq.	*F*-value	*P*-value
PC1	Sex	1	121.10	39.43	**<0.001**
	Temperature	2	1597.60	520.00	**<0.001**
	Localities	3	212.70	69.24	**<0.001**
	Temperature*Localities	3	93.60	30.50	**<0.001**
	Temperature*Sex	3	0.20	0.06	0.937
	Sex*Localities	1	79.50	25.86	**<0.001**
	Temperature*Sex*Localities	3	6.10	1.98	0.064
PC2	Sex	1	2004.50	1594.90	**<0.001**
	Temperature	2	122.00	97.09	**<0.001**
	Localities	3	5.00	3.96	**0.008**
	Temperature*Localities	3	9.50	7.60	**<0.001**
	Temperature*Sex	3	6.10	4.86	**0.008**
	Sex*Localities	1	6.10	4.82	**0.002**
	Temperature*Sex*Localities	3	0.50	0.54	0.771

P values in bold are significantly different at 0.05

The relationship between the diameter of all the eyespots (PC1) and the development time of larvae at each rearing temperature was used to determine the development threshold for each sampling localities (Figure 31). The relationship between PC1 and larval development time has a significant change in slope around a single inflection point for Ako (Davies' test for a change in the slope, $P < 0.001$) and Ndikiniméki ($P = 0.004$). Populations from Mbalmayo and Somalomo also have a significant change in slope though it was not pronounced ($P = 0.02$ and $P = 0.03$ respectively) suggesting that the relationship tended to linearity.

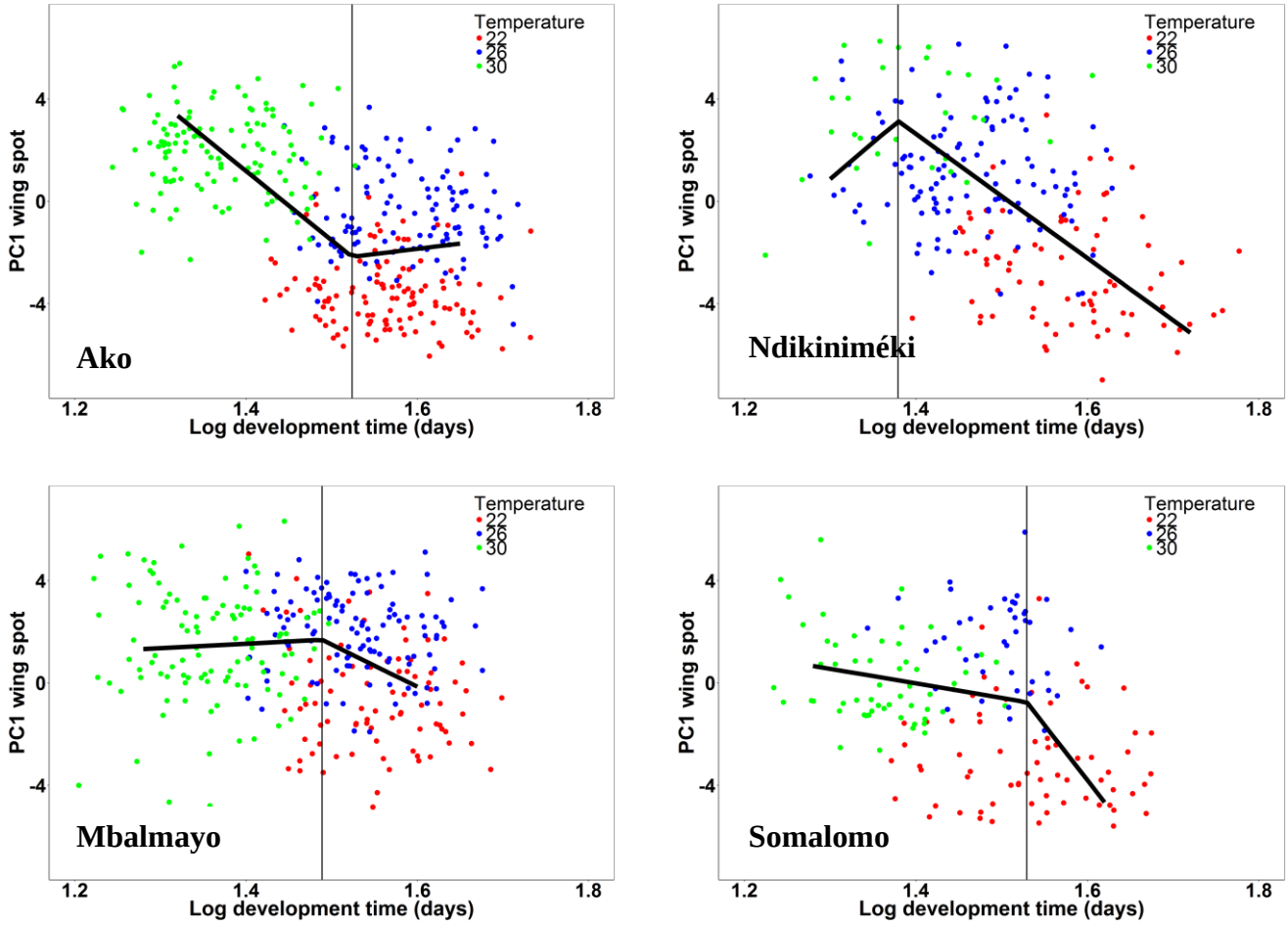

Figure 31. Effects of larval development time on the first principal component (PC1) of nine ventral wing's spots, explaining over 50% of the diameter of eyespot in each population. Graphs represent piecewise linear regressions fitted and explained 45% for Ako, 35% for Ndikiniméki, 5% for Mbalmayo and 14% for Somalomo.

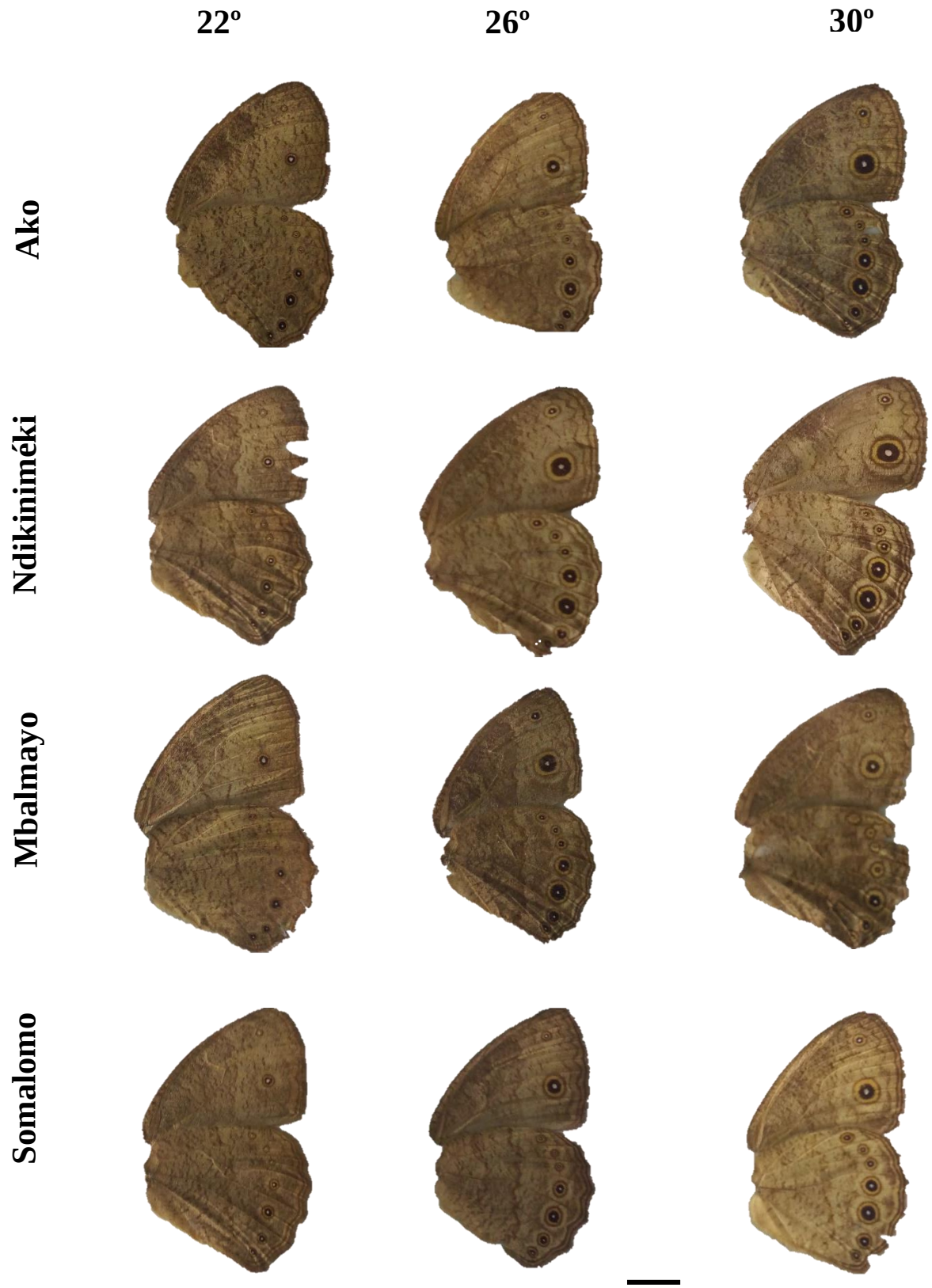

Figure 32. The typical phenotypes of females *B. dorothea* from each sampling localities reared at three constant temperatures in the laboratory. Scale bar: 5cm

III.1.4. Thermal stress variation of *Bicyclus dorothea* across sampling localities and habitat

III.1.4.1. Critical thermal minimum, critical thermal maximum and thermal range

The mean Critical thermal minimum (CTmin), critical thermal maximum (CTmax) and the thermal range (TR) are mentioned in Table X.

Table X. Mean Ctmin, Ctmax and TR of second-generation individuals *B. dorothea* from four localities rear under the same conditions (26ºC, L12:D12 and 75-80%RH).

Habitat	Sampling localities	Sex	Mean CTmin	Mean CTmax	Thermal range (TR)
Ecotone	Ako	Males	4.54 ± 0.21 (n = 59)	45.13 ± 0.14 (n = 59)	40.58 ± 0.28 (n = 59)
		Females	4.60 ± 0.23 (n = 60)	45.11 ± 0.13 (n = 60)	40.51 ± 0.28 (n = 60)
	Ndikiniméki	Males	4.31 ± 0.19 (n = 47)	47.34 ± 0.19 (n = 47)	43.02 ± 0.27 (n = 47)
		Females	4.42 ± 0.20 (n = 50)	46.94 ± 0.20 (n = 50)	42.51 ± 0.31 (n = 50)
Forest	Mbalmayo	Males	4.87 ± 0.22 (n = 45)	44.13 ± 0.21 (n = 45)	39.25 ± 0.30 (n = 45)
		Females	5.15 ± 0.26 (n = 40)	43.25 ± 0.29 (n = 40)	38.10 ± 0.39 (n = 40)
	Somalomo	Males	4.95 ± 0.21 (n = 49)	43.77 ± 0.18 (n = 49)	38.82 ± 0.29 (n = 49)
		Females	5.11 ± 0.21 (n = 49)	43.28 ± 0.25 (n = 49)	38.16 ± 0.29 (n = 49)

Results show significant difference between habitat, but sampling localities and sex did not influence Ctmin. The Ctmax was significantly different between habitat, sampling sites but there was not the effect of sex on this parameter. The same trend was observed for thermal range (Table X, Figure 33). Subsequent Tukey's HSD *posthoc* test performed on all tested parameters show the following trends: Ctmin (Habitat: ecotone < forest; Localities: Ako = Ndikiniméki > Mbalmayo = Somalomo), Ctmax (Habitat: ecotone > forest; Localities: Ndikiniméki > Ako > Mbalmayo = Somalomo; Sex: males > females).

Table XI. Results of the nested analysis of variance (ANOVA) for the effects sampling localities, sex and habitat on the critical thermal minimum, maximum and the thermal range in *B. dorothea*

Trait/Source	Means squares	*df*	*F*	*P*
Critical thermal maximum				
Habitat	574.1	1	292.760	<**0.001**
Sex	16.1	1	8.186	**0.004**
Localities	264.3	3	134.791	<**0.001**
Habitat:Localities	109.8	2	56.007	**<0.001**
Habitat:Localities:Sex	3.1	3	1.585	0.192
Error	2.0	391		
Critical thermal minimum				
Habitat	29.855	1	11.854	<**0.001**
Sex	1.936	1	0.793	0.373
Localities	10.355	3	4.239	**0.005**
Habitat:Localities	1.037	2	0.437	0.646
Habitat:Localities:Sex	0.233	3	0.095	0.962
Error	2.443	391		
Thermal tolerance breath				
Habitat	860.9	1	190.417	<**0.001**
Sex	29.1	1	6.445	**0.015**
Localities	374.9	3	82.931	<**0.001**
Habitat:Localities	132.5	2	29.345	<**0.001**
Habitat:Localities:Sex	5.0	3	1.109	0.345
Error	1767.8	391		

Values in bold are significantly different

The thermal tolerance breadth of each population (Figure 33) was broadly consistent with predicted thermal microclimate variation at each locality, at least with respect to upper thermal limits and maximum temperatures. In contrast, little variation was observed in minimum temperature recorded in the field and the subsequent critical thermal minimum assessed in the laboratory. Overall, the thermal tolerance breadth tended to be broadly more important in Ako and Ndikiniméki than Mbalmayo and Somalomo (Table XI, Figure 33).

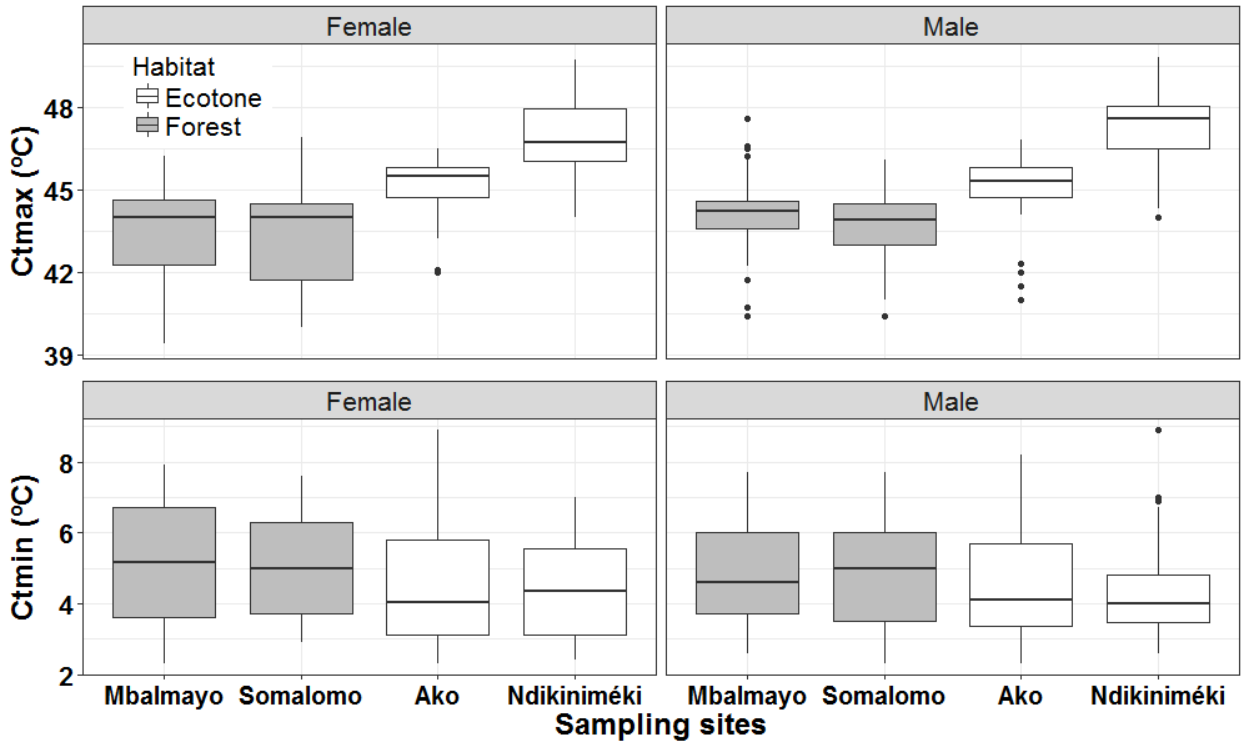

Figure 33. Critical thermal minimum and maximum of different population of *B. dorothea* sampled at different habitats in Cameroon.

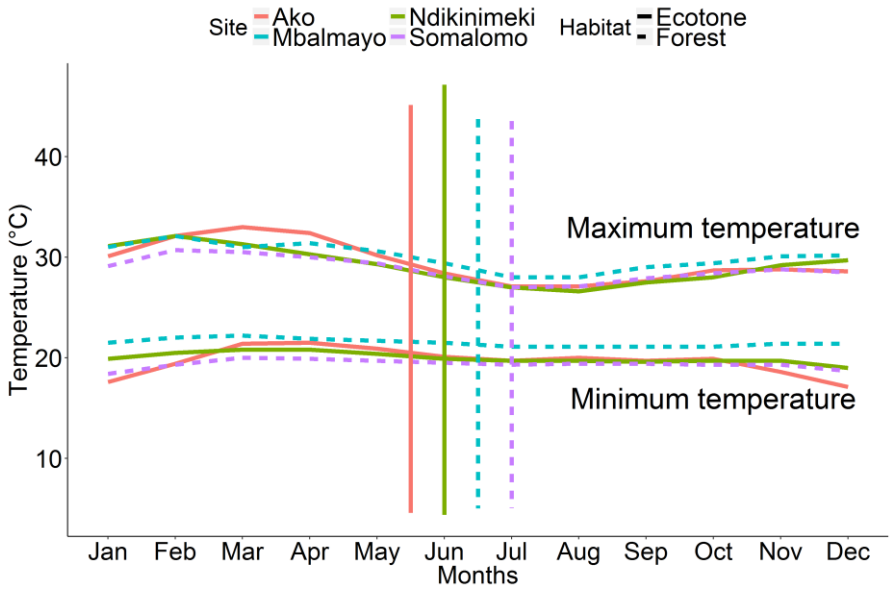

Figure 34. Thermal tolerance breath (vertical lines) for different populations of *B. dorothea* associated with prevailing temperature recorded at each sampling localities in Cameroon.

III.1.4.2. Heat knock down time and recovery time

Mean values of the heat knock down time (HKDT) performed at 40ºC and the recovery time obtained are found in table XII.

Table XII. Mean values of the HKDT and the recovery time after exposure at 40ºC of second-generation adult's *B. dorothea* from four localities

Habitat	Sampling localities	Sex	Mean HKDT (in min)	Mean Recovery time (in min)
Ecotone	Ako	Males	132.80 ± 3.56 (n = 41)	29.92 ± 1.71 (n = 41)
		Females	152.39± 3.08 (n = 31)	31.93 ± 1.86 (n = 31)
	Ndikiniméki	Males	147.69 ± 7.80 (n = 29)	41.38 ± 7.28 (n = 29)
		Females	171.05 ± 8.62 (n = 36)	47.88 ± 5.84 (n = 36)
Forest	Mbalmayo	Males	122.93 ± 4.80 (n = 45)	51.27 ± 4.25 (n = 45)
		Females	129.02 ± 6.27 (n = 47)	50.75 ± 5.16 (n = 47)
	Somalomo	Males	93.38 ± 2.27 (n = 44)	34.91 ± 3.16 (n = 44)
		Females	96.96 ± 3.45 (n = 28)	43.28 ± 4.96 (n = 28)

The heat knock-down time was significantly affected by sampling localities, habitat, sex and the interaction between habitat and sampling localities (Table VIII). The *post hoc* HSD test shows that population from Ndikiniméki spends more minutes under the fixed temperature (40º0) before being knock down (Ndikiniméki > Ako > Mbalmayo > Somalomo). When considering vegetation structure, ecotone populations were more heat-resistant than forests' ones (Ecotone > Forest) and finally females were more heat-resistant than males (Figure 35).

The time needed by knocked butterflies to recover at 25ºC was also affected by sampling localities and habitat but not within sex (Table XIII) with butterflies from Mbalmayo spending more time in their apparent coma before they recover. The HSD *post hoc* test gave the following trend (Mbalmayo^{a}, Ndikiniméki^{ab}, Ako^{bc}, Somalomo^{c}).

Table XIII. Effect of habitat, Sex and Sampling localities on the heat knock down performed at 40ºC and the subsequent recovery time at 25ºC after the heat knock down.

Trait/Source	Means squares	*df*	*F*	*P*
Heat knock down				
Habitat	108474	1	98.577	**<0.001**
Sex	11520	1	10.469	**0.001**
Localities	26166	3	23.779	**<0.001**
Habitat:Localities	22981	2	20.884	**<0.001**
Habitat:Localities:Sex	1696	3	1.542	0.204
Error	1100	293		
Recovery time				
Habitat	4588	1	5.972	**0.015**
Sex	997	1	1.298	0.101
Localities	6767	3	8.807	**<0.001**
Habitat:Localities	6228	2	8.106	**<0.001**
Habitat:Localities:Sex	320	3	0.417	0.740
Error	768	293		

Values in bold are significantly different at 95%

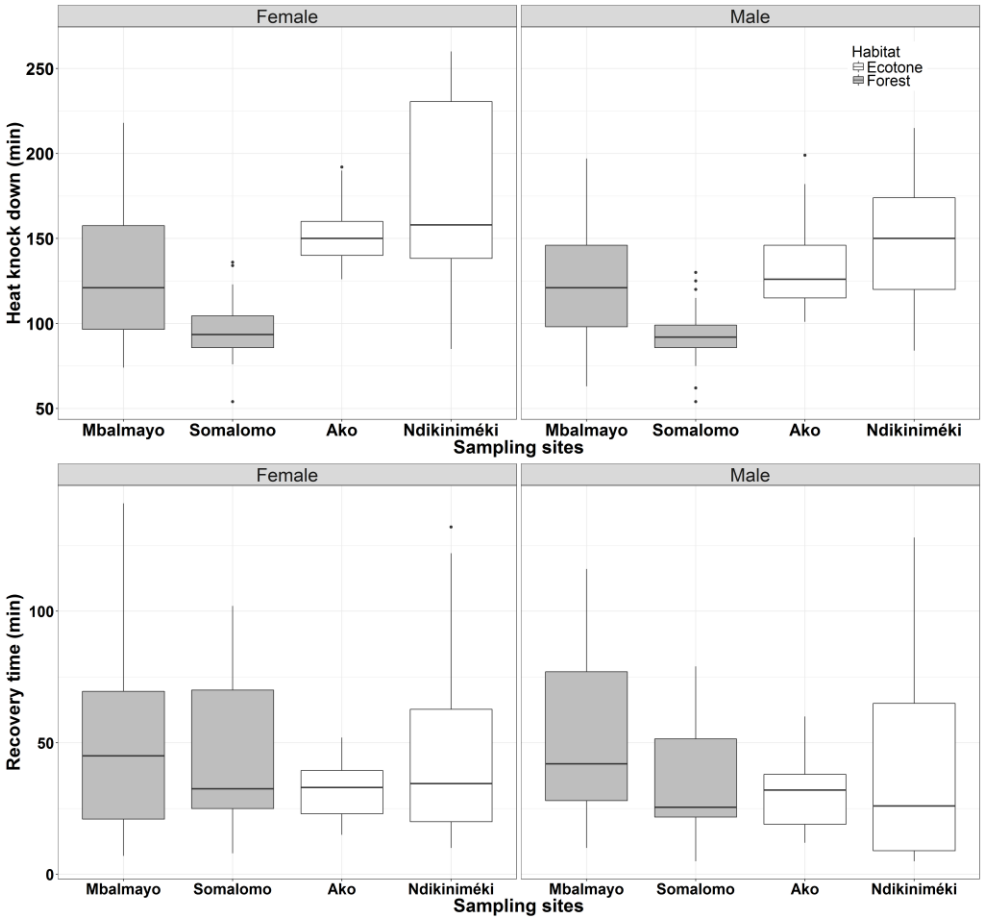

Figure 35. Heat knock down and heat knock down recovery times of second-generation adults *Bicyclus dorothea* populations sampled at four localities representing two habitats in Cameroon.

III.2. Discussion

The present work focuses on the mechanisms of adaptation to different climates, temperature, of different populations of the light bush brown butterfly, *Bicyclus dorothea* found in various habitats in Cameroon. A brief bio-ecological description of the butterfly itself was also provided since this information was lacking. The thesis takes an integrated approach to thermal adaptation and brings together studies at the phenotypic and physiological levels. By examining geographical variation among wild populations, this work investigates how *B. dorothea* is adapted to different habitats with varying thermal conditions. Furthermore, the thesis investigates how *B. dorothea* populations cope with alternating wet and dry seasons at each of the localities where the study was carried out. Because of the major influence of temperature on the ecology and evolution of species, the way organisms adapt to thermal variation has long captivated the attention of biological research (Parmesan, 1996 and 2006; Walther *et al.,* 2002; Kingsolver *et al.,* 2011). In recent years, the field of thermal adaptation has seen a surge of interest because of the observed and predicted impact of recent climate change on biodiversity. Moreover, recent climate change is predicted to have severe impacts in tropical zones compared with other regions of the world (Deutsch *et al*., 2008; Huey *et al*., 2009). The results presented in this thesis contribute to our general knowledge of the mechanisms of adaptation to environmental variation in ectotherms with *B. dorothea* as a model species.

III.2.1. Bio-ecology of *Bicyclus dorothea*

III.2.1.1. Morphology of developmental stages

The developmental stages of *B. dorothea* globally resemble those of almost all butterfly's species with a classic eggs, larvae, pupae and adults. This study shows that *B. dorothea* develops into five larval stages, that can be easily distinguished morphologically. This is one of the characteristics of species belonging to the genus *Bicyclus* (Condamin, 1973; Sourakov and Emmel, 1997). However, there are morphological differences that can be observed in all instar. For example, larvae are always green in *B. dorothea* while in *B. vulgaris* and *B. safitza*, they turn into dark brown at the fourth larval instar (pers. obs) and into light brown in *B. anynana* (Brakefield *et al*., 2009). This difference in larval color among species is well known in lepidoptera and is mainly due to the level melanism (Välimäki *et al*., 2015).

Morphological measurements of male and female's wing patterns show distinctive variation between sexes. Males are smaller in size than females regarding traits like wing length and width and

body length. This is a common observation found among the members of the genus (Condamin, 1973; Larsen, 2005). Also, the diameter of the wing spot was reduced in male than in female. One of the most visible wing features in *Bicyclus* is the presence of wing spots. Many *Bicyclus* species display strong phenotypic variation of these traits over seasons (Brakefield and Reitsma, 1991; Windig *et al*., 1994, Roskam and Brakefield, 1999). *Bicyclus dorothea* adults have among the lightest colored eyespots of the group, although the species is very similar to other members of the "*dorothea*-complex", namely *B. moyses* and *B. jefferyi*. *Bicyclus dorothea* possesses two eyespots on each of the forewings and seven on each of the hind wings, although sometimes individuals can display three eyespots on the forewing and eight on the hind wing. The underside of the wing is gray beige with the eyespots not visible. The male's hind wing bears two androconial brushes which are absent in females; this is one of the main features that can be used to differentiate *B. dorothea*, *B. moyses* and *B. jefferyi*. In fact, males of all three species have two androconial brushes in cell Rs and CuA2 of the hind wings, but in *B. dorothea* the brush is light brown to yellow and weakly developed in cell Rs while in *B. moyses* the brush is darker, and it is almost black in *B. jefferyi* (Aduse-Poku *et al*., 2017).

III.2.1.2. Life history traits

Results obtained on few life histories traits such as the development time of immature stages did not reveal important differences between sexes. While in *B. anynana* protandry is sometimes observed (Zwaan *et al*., 2008), it was not the case in this study. The development time of eggs, larvae and pupae was not significantly different between sexes. However, the duration from eggs to the death of adults was twenty-days longer in females than in males under laboratory conditions. This simply means that males die earlier than females with phenological consequences such as the timing of reproduction before the onset of dry season which is known to be challenging in *Bicyclus* butterflies (Brakefield and Reitsma, 1991). Also, pupal weight varied between males and females, which likely due to reproductive trade-off in both sexes. Reproductive activities of females (eggs laying) might be the cause of such variation because they need more food resources than males. Hence, females' larvae will tend to accumulate the maximum of food leading to heaviest pupae.

III.2.1.3. Geographic distribution in Cameroon

The survey conducted in thirty-six localities in seven regions revealed the presence or the absence of *B. dorothea* in some of these localities. Overall, *B. dorothea* was present in low to mid-altitude localities characterized by the presence of different forest types with relatively stable

temperatures throughout the year. In the savannas and other opened areas, it is quite impossible to find individuals. This suggests that the species does not like long exposure to the sun which might lead to physiological disturbances due to heat accumulation, unlike species like *B vulgaris*, and *B. safitza* that can be found in open areas (pers. obs). The thermal niche of *B. dorothea* makes it particularly sensitive to environmental variations which might be the reason why the species appears to favor degraded or disturbed forests microhabitats. Moreover, the presence of the species is eventually favored by the presence of larval host plants of the family Poaceae, and the adult food which is composed by fallen fruits such as those from the umbrella tree *Schefflera actinophylla*, mango *Mangifera indica*, goyava *Psidium goyava* and many others.

III.2.2. Seasonal polyphenism in wing pattern across localities in the wild

The results presented in this thesis demonstrate that sampling locations, habitat and season have strong effects on wing pattern elements in *B. dorothea*. Among all wing characters studied, the diameter of eyespots was highly sensitive to the effect of sampling localities, habitat, sex, seasons and the interaction between these parameters. All populations examined in this study show a certain degree of plasticity with respect to the diameter of their eyespots. This variation is strongly seasonal and more pronounced in populations belonging to ecotone habitat (Ako and Ndikiniméki) than those found in the forest (Mbalmayo and Somalomo). Generally, there are three important patterns in seasonal polyphenism in *B. dorothea*: 1) the diameter of the wing spot which is the morphological trait undergoing plasticity the most in *B. dorothea* as in many other *Bicyclus* species; the length of the wings were not affected by the fixed parameters; 2) a general observation indicating that when taking into account the prevailing habitat, ecotone populations tend to be more variable than their forest counterparts; but when considering each of the sampling location, population from Ako was more seasonally plastic than Ndikiniméki while butterflies from Mbalmayo were more plastic than those from Somalomo; 3) change in eyespot size occurs seasonally suggesting an adaptation of these populations to alternating changes in climate annually in the wild (Brakefield and Larsen, 1984; Brakefield and Reitsma, 1991; Brakefield *et al*., 2007).

Previous studies on seasonal polyphenism in *Bicyclus* butterflies in a single locality in Malawi showed that seasonal forms of these butterflies differ drastically in color and wing pattern elements over the wet and dry seasons (Windig *et al*., 1994). A similar example was also found in Australia where butterflies belonging to the genus *Mycalesis* Hübner, 1818 exhibit the same pattern of plasticity of their wing elements over seasons (Braby, 1994). This plastic response in *Bicyclus* butterflies to wet

and dry seasons was shown to be directly related to changes in environmental components (Brakefield and Reitsma, 1991; Windig *et al*., 1994; Brakefield and Zwaan, 2011). The role of temperature was highlighted as the main factor inducing these different morphs (Roskam and Brakefield, 1996; Brakefield *et al*., 2007) though biotic factors like food plant quality can also influence the development of wing patterns, and hence can drive plastic responses in the field (Kooi *et al*., 1996). One of the key climatic characteristics of ecotones is the greater annual variation in environmental variables (Longman and Jenik, 1992). In these regions, while rainfall patterns are generally unimodal with important differences between day and night temperatures (Hirota *et al*., 2010; Ibanez *et al*., 2013), tropical rainforests are often characterized by a bimodal rainfall pattern, with relatively small differences between day and night temperatures.

Brakefield and Reitsma (1991) highlighted that temperature decreases during the dry season in a unique study area in Malawi was the principal cue leading to the dry season form (with reduced wing spots) of five *Bicyclus* species in the wild. The strong plasticity observed at Ako and Ndikiniméki localities during the dry season can be explained by the heterogenous nature of the environment. These localities have a particular physiognomy which is a mosaic of forest fragments surrounded by tree-less savanna (Ndikiniméki) and sub-montane forest and upland savannah (Ako). In the other hand, forest habitats are more homogenous though Mbalmayo shows signs of a degraded forest in contrast with Somalomo where anthropogenic activities are not more developed since it is a protected area. These physiognomic differences have consequences on the climate pattern prevailing at each locality and hence on the physiology of insects that inhabit them. Together, vegetation patterns and temperature fluctuations increase environmental heterogeneity at the microhabitat level. For instance, in regions with a unimodal rainfall pattern, dry seasons tend to be longer and drier than those of the regions with bimodal rainfall patterns (though this also depends on altitude), leading to drier environments in ecotones during the dry season compared to forest environments (Tsalefac *et al*., 2003; see Figure 36). Hence, while tropical rainforests have almost constant mean monthly temperatures and availability of larval host plant throughout the year, larval host plants are likely only available in the wet season in ecotone habitats where there is considerable fluctuation in mean monthly temperatures over seasons (Chan *et al*., 2016). Predation very likely plays an additional key role in maintaining wing spot plasticity. Investigations on the role of predation in driving wing spot plasticity over seasons have not yet been done in *B. dorothea*. In *B. anynana* however, changes in wing patterns are known to be adaptive in that dry season forms are cryptic while conspicuous

eyespots in wet season forms can contribute to deflection of and survival from predator attacks (Lyytinen and Brakefield, 2004; Prudic *et al*., 2014).

It is well known that behavioral responses to climate variation are among the most immediate responses of animals in general and butterflies in particular (Walther *et al*., 2002; Parmesan, 2006). Two strategies can be used by many Satyrine species: 1) adults can cease their reproduction during unfavorable dry seasons and females will start reproducing at the onset of the rainy season (Brakefield and Larsen, 1984), or 2) persisting adult females during the dry season will move into an aggregate of moist refugia in the understory where they will sometimes lay their eggs on the few remaining fresh host plants for larval growth (Braby, 1995; see Figure 36). Larvae from these eggs will then develop in an environment with low temperatures (as eggs were laid in moist refugia) and will likely yield adults with reduced spots. The high plasticity observed in Ako and Ndikiniméki populations suggests adaptation to complex changes such as lack of larval food plants and predator avoidance, occurring during the dry season but the mechanisms behind these patterns require further studies. Exploring seasonal polyphenism in life history traits such as reproductive diapause, egg fertility, population dynamics of *Bicyclus* species across these localities (ecotone vs forest) will provide insights into such evolutionary aspects of phenotypic plasticity.

III.2.3. Temperature-induced plasticity in different populations of *Bicyclus dorothea*

An important way to assess an organism's plastic response is the analysis of the reaction norm, i.e. the phenotypic expression of a genotype across an environmental range (Pigliucci, 1996). By measuring the reaction norms of geographically different populations under the same range of conditions (common garden approach), the level of genetic divergence between the populations for the plasticity response can be determined. A difference in the slope or shape of the reaction norm corresponds to a genetic difference underlying the plastic response to environmental conditions (genotype–environment interaction). In this part of the study we investigated habitat variation for seasonal plasticity in *B. dorothea* by comparing thermal reaction norms for few life history traits and wing pattern elements of four populations from two habitat types. The main issue to address behind this session was to show any evidence of local adaptation of *B. dorothea*'s populations to specific local climates or whether the same plastic response is expected from these populations. In the light of climate change this question is relevant because, when predicting species' response to climate change, it is important to understand to what extent phenotypic plasticity allows organisms to cope with

changing temperatures. The more specialized is an organism in its phenotypic plasticity response to temperature, the less likely it will successfully cope with changing climatic conditions.

Figure 36. Pictures showing the change in the physiognomy of *Bicyclus dorothea*'s microhabitats across seasons in the wild in two selected sampling locations. Somalomo: a) wet season; b) dry season; Ndikiniméki: c) wet seasons; d) dry season.

III.2.3.1. Life history traits

The results presented in this thesis clearly show that rearing temperature can induce important differences in selected life history traits of *B. dorothea* though in the wild they are complex environmental interactions/cues to which populations are exposed. Generally, development time of immature stages of all the four populations tested decreased with increasing temperature; this is a general rule well known in ectotherms and which is not an adaptive plasticity (Gotthard *et al*., 1994 and 1995; Nylin and Gotthard, 1998). Apart from larvae, no significant difference in development time was observed in the other immature stages of all the four populations studied. The shape of the reaction norm of development time of larvae of the populations show different patterns across rearing temperatures. At 26 and 30ºC, the development time was almost the same for all populations but at 22ºC, there was clear difference. While larvae from Ako and Mbalmayo had almost the same development time, those from Ndikiniméki showed more prolonged development (mean: about 34 days) and those from Somalomo a more delayed development (mean: about 27 days). Interestingly, these results were obtained from second-generation individuals from the laboratory stock reared under the same conditions (26ºC, 75-80%RH and L12:D12) and the same host plant (*Axonopus compressus*). The plasticity observed at 22ºC for the development time of larvae is very informative as it is the most active immature stage, and which can also react actively to environmental change through behavioral modifications like moving to a colder/warmer place or which can change host plant etc. This result suggests an adaptative response to low temperatures of all the populations studied as they are from different environmental conditions. In fact, temperature and rainfall data recorded at each sampling localities revealed important differences in those climatic factors. Regarding these climatic patterns, Mbalmayo, Somalomo and Ndikiniméki exhibit a bimodal rainfall while Ako is dominated by a monomodal rainfall pattern. Further, temperature profiles of the localities show more elevated temperatures during the dry season at Ako compared with the other localities. It is well known that rainfall and temperature pattern are important plasticity-driven factors in Satyrine butterflies (Windig *et al*., 1994; Brakefield and Zwaan, 2011). The long exposure of different populations under such conditions in the wild likely have led to local adaptation which is heritable from one generation to another.

Pupal weight as the development time of immature stages also varied across rearing temperatures. While there was a trend showing increasing weight with decreasing temperature for all populations, these differences were also significant among populations at each rearing temperature. In both males and females, populations belonging to forest habitat (Mbalmayo and Somalomo) have

the heaviest pupae compared with their ecotone (Ako and Ndikiniméki) counterparts at all rearing temperatures, even though they were all given the same larval host plant during laboratory experiments. Females were heavier than males, a difference well known among the invertebrates because reproductive activities in females in term of energy costs more than in males (Atkinson, 1994; Boggs, 1997a and b; Bonebrake *et al*., 2010). In females, pupal mass was continuous across temperatures for Ndikiniméki and discontinuous for the remaining localities while in males, the discontinuity of the reaction norm was more pronounced for Ako and Ndikiniméki. Furthermore, the ANOVA model indicated a significant interaction between rearing temperature and sampling localities. These results indicate a plastic adaptation of these populations to their local habitat. In fact, pupal weight is a combination of at least two important environmental factors: the developmental rate which is itself highly correlated with temperature, and food quality (Kooi, 1992; Kooi *et al*., 1996; Fischer *et al*., 2004; Saastamoinen *et al*., 2013).

During development from zygote to metamorphosis, the tissue of an organism differentiates and expands through a sequence of cell divisions and cell growth. This differentiation is made by a set of physiological and biochemical processes that are enzyme-dependent which themselves depend on the temperature under which these processes are ongoing (Atkinson, 1994; Angilletta *et al*., 2004). As a rule in ectotherms, individuals from cooler environments often exhibit larger sizes at maturity than those from warmer environments (Atkinson, 1994). In fact, lower temperatures slow maturation due to their impact on enzymes' activities but allow the accumulation of important quantities of nutrients which will lead to individuals with relatively important weight. On the other hand, warm temperatures accelerate enzymes' activities, facilitating all metabolic reactions that lead to early maturation of individuals.

Food quality is also an important factor that can have effect on pupal weight in Lepidoptera (Boggs, 1997b; Fischer *et al*., 2004). When food quality is high, larvae will slow maturation thereby increasing storage reserves of nutritional resources so that adult fecundity will be higher. If food quality is poor, larvae can employ one of three strategies (Stearns and Koella, 1986): (1) increase development time to gather enough resources to attain normal adult size by decreasing growth rate, (2) maintain normal development time but attain smaller adult size with fewer reserves, again by decreasing growth rate, or (3) decrease development time but become a smaller adult without changing growth rate. Considering these results and the previous explanations, one might suspect that populations inhabiting forests live in an environment with relatively stable and warm temperatures throughout the year, with abundant availability of larval host plant regardless of season, is the reason

for producing heaviest pupae, which is a proxy of adult's body size. On the other hand, populations from ecotone likely have the challenge of larval host plant scarcity during the harsh dry season combined with high temperature fluctuations across seasons might have serious impacts on life history traits and hence on adult's fitness. In *Bicyclus* butterflies, the reduction in body mass during ontogeny can lead to females with limited reproduction as a trade-off to larval food allocation (Fischer *et al*., 2004; Saastamoinen *et al*., 2013). It is likely the reason why in *B. anynana* reproduction ceases during the dry season in East Africa due to the lack of larval food plant (Zwaan *et al*., 2001, Brakefield and Zwaan 2011), while in the rainforest species *B. sanaos*, reproduction continues throughout the year (Oostra *et al*., 2014; Brakefield *et al*., 2014).

However, caution must be considered for these differences between populations in terms of adaptive differentiation because these traits might have been influenced by population-specific differences in food plant adaptation. Also, seasonal variation in life history traits is a combination of numerous cues (Shapiro, 1976; Roskam and Brakefield, 1999) that have not been investigated here. Furthermore, endocrine regulation plays a key role in accomplishing such phenotypic integration of multiple traits and adjusting them in a coordinated and timely fashion (Brakefield *et al*., 1998, Geister, 2008). In *B. anynana* for instance several seasonally plastic traits are controlled by a single hormonal system (Oostra *et al*., 2011; Hartfelder and Emlen, 2012; van Bergen *et al*., 2017). The most active group of hormones known in insects to date is Ecdysteroid hormones. They control molting, pupal development and mediate developmental plasticity of the ventral wing pattern (Koch *et al*., 1996; Brakefield *et al*., 1998), female relative abdomen size, and female reproductive strategy (Oostra *et al*., 2014; Brakefield *et al*., 2014).

III.2.3.2. Wing pattern

In adult butterflies, phenotypic plasticity is generally expressed as the variation in wing morphology (Brakefield and Larsen, 1984). Summarizing the PCAs and the ANOVA, it can be concluded that variation in rearing temperature has important effects on some wing pattern elements in *B. dorothea*. The same trend has been recorded in previous investigations of other *Bicyclus* species like *B. anynana, B. ena, B. safitza, B. cotrelli* and *B. vansoni* (Roskam and Brakefield, 1996; Nokelainen *et al*., 2017; van Bergen *et al*., 2017). The first two principal components (PC1 and PC2) accounted at least for 75% of the total variation in wing morphology in all populations. The diameter of the nine wing spots loaded mainly on PC1 with at least 52% while the length and the width of the wings loaded heavily on PC2.

The analysis of variance applied to PC1 and PC2 revealed a strong effect of rearing temperature, sampling localities and their interaction confirming that all characters loading on these principal components are temperature-dependent and the existence of a phenotypic plasticity of wing pattern to development temperature in *B. dorothea*. However, the analysis of the reaction norm of PC1 shows variations along the temperature gradient, resulting in important differences regarding the diameter of the wing spots among populations also between sexes. In all populations, individuals reared at low temperature developed wings with reduced eyespots in both males and females while at high temperature, the eyespots were prominent. Differences were observed among populations at a given rearing temperature, indicating different responses to temperature gradient in all populations studied. For example, at 22ºC populations from Mbalmayo show more enlarged eyespot than the other remaining populations. Also, regarding the reaction norms, no important variation in wing spots was observed in populations from Mbalmayo between 26 and 30ºC in both males and females.

Similarly, the body size (PC2) was inversely related to temperature though there was not a perfect linear relationship; low temperature yielded larger individuals in all populations and vice versa. There were also sex differences in body size, which were larger in females than in males. Males and females showed little inter-population differences for PC2, resulting in overall slightly higher values for the Mbalmayo population than Ndikiniméki in both males while in females, Ako displayed the highest mean PC2 value and Ndikiniméki has the lowest.

From the analysis of the reaction norms, populations reacted differently to temperature gradient at the level of their wing morphology even though wing measurements were carried out on the second-generation individuals. Further, the reaction norms of all populations studied did not show linearity with temperature range suggesting the polyphenic nature of *B. dorothea* as demonstrated in the seasonal variation of wing pattern in different localities in the wild. Previous studies illustrated similar findings in a South African and a Malawian populations of *B. anynana* (De Jong *et al*., 2010; Nokelainen *et al*., 2017) and in 10 latitudinal populations of *Drosophila melanogaster* in Peru (Land *et al*., 1999). These differences in wing patterns in response to temperature gradients are indicators of adaptation of these populations to local environment. In fact, our results show that populations inhabiting forests were less plastic considering the diameter of the wing spots than those of localities situated in ecotones. These results are consistent with Oostra, Brakefield *et al*. (2014) who demonstrated that due to the adaptation to non-seasonal environment, *Bicyclus martius sanaos* (Fabricius, 1793) does not undergo high plasticity as *Bicyclus* species inhabiting more seasonal environment. The seasonal variation of wing spot in most *Bicyclus* species is an adaptive strategy to

cope with alternative wet and dry seasons in their native habitats (Brakefield and Reitsma, 1991; Windig *et al*., 1994; Brakefield and Zwaan, 2011). In *B. anynana*, wing spots play a role in deflecting attacks by predators (Lyytinen and Brakefield, 2004). However, the degree of plasticity is a function of the quality of the cue inducing it (Roskam and Brakefield, 1999). These reasons can explain this differential plasticity observed during this study. Climatic data of ecotone localities (Ako and Ndikiniméki) show the contrasting environments of the alternative wet and dry seasons. On the other hand, forest localities (Mbalmayo and Somalamo) are more homogenous than those in the ecotones in climatic characteristics. Forests are not then exposed to harsh dry season conditions where being highly plastic will have an impact on their fitness (Shapiro, 1976; West-Eberhard, 1989; Westerman and Monteiro, 2016). Even though temperature represents an important factor affecting the biology of cool blooded animals, results obtained under laboratory conditions must be taken with caution because other cues can drive plastic responses in ectotherms (Shapiro, 1976; Pigliucci, 1996; Roskam and Brakefield, 1999). For example, food quality has been shown to have effects on wing morphology in *B. anynana* (Kooi, 1992; Kooi *et al*., 1996).

III.2.3.3. Relationship between larval development time and wing pattern

Results presented in this thesis show a correlation between larval development time and wing spots (PC1) in all populations studied. Further, there was a single inflexion point for all the piecewise regression performed for all populations across developing temperature confirming the polyphenic status of *B. dorothea*. The piece-wise regression curves show different patterns among populations. This result is consistent with our predictions and those found in other *Bicyclus* species inhabiting seasonal and non-seasonal environments (Nokelainen *et al*., 2017; van Bergen *et al*., 2017). However, the relationship between development temperature and PC1 differs from one population to another, strengthening the hypothesis that any environmental factor affecting pre-adult development could act as a morph-determining predictable cue (Brakefield and Frankino, 2009). Many factors other than temperature are known to affect adult morphology through exposure of immature stages. For example, food limitation during larval development in *B. anynana* and a reduced quality of food are associated with longer larval development time, yielding dry season morph (with reduced wing spot) regardless of the value of development temperature (Holloway *et al*., 1991; Kooi, 1992; Kooi *et al*., 1996; Saastamoinen *et al*., 2013).

The developmental plasticity observed in larvae and the subsequent repercussions on wing pattern indicate that natural populations of *B. dorothea* contain enough genetic divergence in response

to thermal variation for developmental plasticity. It is however well known that developmental plasticity is mediated by hormone signaling (juvenile hormone) and enzyme activities which are also temperature-sensitive in many insects' species (Atkinson, 1994; Nijhout, 2003a; Davidowitz and Nijhout, 2004; Hartfelder and Emlen, 2012). The thermally sensitive traits shown here are likely to have involved evolutionary changes in the timing of larval development via hormone titers in response to external cues (temperature here) or in the degree and timing of the sensitivity of hormonal receptors in the developing target tissues which in turn has subsequent consequences in wing morphology (Nijhout, 2001 and 2003b; Oostra *et al*., 2014; Mateus *et al*., 2014). Local climates can be therefore important evolutionary drivers affecting populations in different ways, explaining the morphological responses observed in the wild during this study. These results are also important in the way that ongoing climate modifications especially in the tropics, will likely affect ectotherms populations differently in the future.

III.2.4. Thermal stress adaptation in different populations of *Bicyclus dorothea*

III.2.4.1. Variation in Ctmin and Ctmax

Consistent with predictions, the Ctmax was higher (by ~3ºC) and Ctmin was lower (~1ºC) for second generation *B. dorothea* individuals originating from the variable ecotone habitats relative to forest individuals. These results suggest that the habitat-specific thermal limits observed for this tropical ectotherm, and the upper thermal limits, have a genetic basis and are not a consequence of acclimation or developmental plasticity. Our conclusions corroborate recent findings that tropical ectotherms have limited tolerance to warming (García-Robledo *et al*., 2016) and limited ability to plastically respond to climate change (Gunderson and Stillman, 2015). We further suggest that tropical forest species might be especially vulnerable to ongoing warming climate.

Acclimation and plasticity are vital to understand organismal responses to climate change (Somero, 2010). While we did find different thermal limits across habitats for populations reared under shared environments, all colonies were reared at a single temperature (26ºC). The magnitude of responses of all populations to heat stress may have been less differentiated if populations were acclimated to a warmer temperature. Similarly, we may have found a larger difference in cold tolerance if populations have been acclimated to colder temperatures. Furthermore, heat hardening (short-term acclimation to warm temperatures) has been demonstrated in *Bicyclus anynana* (Fischer *et al*., 2010), indicating that for this study *B. dorothea* could have exhibited more short-term plastic responses to thermal variation if the heat hardening was applied before the assessment of thermal

tolerance. Ultimately, insect responses to climate change will be the result of a complex interplay between behavior, phenology, evolution and plasticity in response to thermal variation across multiple temporal scales (Bonebrake *et al*., 2014; Kingsolver and Buckley, 2017).

The variation in Ctmin and Ctmax observed in our populations raise important questions on the origin of such differential responses to thermal stress in populations of the same species across habitat. Many factors including the environment experienced by individuals in the wild, their physiology, behavior and genetic composition are likely involved in thermal response of ectotherms (Walther *et al*., 2002; Parmesan, 2006). The genetic mechanisms underlying the response of ectotherms to thermal stress are known to be mediated by the heat shock proteins (HSPs) which can act in different timescale (Denlinger and Yocum, 1998; Sørensen *et al*., 2003). These HSPs are of different categories and their production in ectotherms is induced by heat and other stresses (Pockley, 2003; Sørensen *et al*., 2003). For widely distributed ectotherm populations occurring in a wide distribution area with different climate patterns, it is possible that the expression of genes coding to produce HSPs can be affected (Colinet *et al*., 2010; Rolandi *et al*., 2018). Further, these gene expressions could have been transmitted and conserved in F2 offspring established under laboratory conditions of our experiment (Mousseau and Dingle, 1991; Mousseau 1998). Such results have been found in the production of the HSP70 in different population of the of the Cooper butterfly, *Lycaena tytirus* (Karl *et al*., 2009) and in *Drosophila* species (Jenkins and Hoffmann, 1994b; Rolandi *et al*., 2018). The temperature variation recorded at each locality (figure 34) shows different patterns with low variation in minimum values but with important variation in maximum temperatures. It is possible that this temperature variation experienced by wild populations of *B. dorothea* could have impacted gene expression for HSPs and hence could explain the different response to heat stress observed in this study.

III.2.4.2. Variation in heat knock down and recovery times

It is well known that climate and especially temperature exerts a strong influence on insects' distribution and abundance (Wilson *et al*., 2005). The sequence of thermal events leading an insect to immobility or death occurs at a narrower range at high than at low temperatures. (Hazell *et al*., 2010). The upper temperature at which an insect loses the ability to move is known as the heat coma temperature and it is generally followed by the lethal temperature. Some insects can recover from heat coma temperature depending on the duration of exposure, but it sometimes leads to their death (Folk *et al*., 2006). In this part of the study, we compared the ability of second-generation adult

butterflies from the four sampling localities (hence habitat) to withstand exposure at 40ºC and to recover at 25ºC after their heat coma. The results demonstrated that individuals from Ndikiniméki and Ako (ecotone) spent more than two hours (150 min) at 40ºC before the onset of heat coma while those from Mbalmayo and Somalomo succumbed to heat coma at ~120 min ~90 min respectively. Moreover, the recovery time after the knock down at 40ºC shows little but significant differences among populations with few dead individuals recorded. The salient information from these results is that all populations tested responded to heat exposure differently. This is a tendency well known in ectotherms species sharing different habitat in their geographic distribution (De Jong *et al*., 2010; Phillips *et al*., 2016; Yuan *et al*., 2018) because the climate in the environment they experienced during ontogeny is often different from subsequent development and can have repercussions on their ability to withstand artificial climate imposed in the laboratory. These results are consistent with previous findings in *B. anynana*, a congener of *B. dorothea* (Fischer *et al*., 2010). The heat coma and the recovery time values are ecologically important when investigating the effect of climate warming on insects because they represent the upper thermal limit which can lead to the death of the animal (Angilletta, 2009). Hence, it gives crucial information on the ability of different populations inhabiting distinct habitats to cope with predicted future climate warming in the tropics (Deutsch *et al*., 2008). However, a fundamental question we can ask ourselves is the extent to which laboratory results obtained here can accurately predict negative effect of climate warming under field conditions. The answer to this question is a cautious "yes". Firstly, the maximum temperature recorded in 2016 at each of the localities were 36.42, 35.36, 34.01 and 34.63ºC for Ako, Ndikiniméki, Mbalmayo and Somalomo respectively. These values were recorded during the dry season and happened just two or three times throughout the year representing occasional peak in temperature which can eventually pose a challenge to ectotherms species. Moreover, these values were not too far from the temperature (40ºC) at which heat coma was assessed, pointing the fact that warming events are already happening in Central Africa because two decades before, such records did not occur (Runge, 2007). Secondly, the exposure duration to heat in the laboratory ranged from 90 minutes to more than 150 minutes, a situation which is rare under natural conditions regarding the duration (~ 30 to 60 minutes) of these high temperatures collected at each locality (IITA, unpublished data). Also, through climate warming, tropical insects are likely to experience high temperatures in the future; for example, mean temperature is expected to rise by about 1ºC in 2050 in the tropical rainforests (Pachauri *et al*., 2015). The caution generated from this result is that, under natural conditions there is microclimate heterogeneity that can help buffering the detrimental effects of heat (Bonebrake and Deutsch, 2012).

Moreover, immature stages of *B. dorothea* were not tested and were reared under a unique temperature (26ºC), limiting the potential role of developmental plasticity in structuring heat coma response in adults *B. dorothea* (Fischer *et al*., 2010; Phillips *et al*., 2016; Llewelyn *et al*., 2018). However, the results obtained here give a clear idea on how different populations of insects can be affected by high temperature or climate events like heat waves in tropics (Fischer *et al*., 2014).

CONCLUSION, RECOMMENDATIONS AND PERSPECTIVES

Conclusion

In this thesis we wanted to determine how insects deal with the challenges linked to environmental variations, mainly temperature across different habitat types and the strategies they use to withstand these challenges. We used some bio-ecological aspects of the widely distributed light bush brown butterfly *Bicyclus dorothea* in Cameroon as a model for this investigation. Specifically, the thesis takes an integrated approach to thermal adaptation and brings together studies at the phenotypic and physiological level. By examining geographical variation among wild populations, the work investigates how *B. dorothea* is adapted to geographically varying environmental conditions over seasons. Because of the major influence of temperature on the ecology and evolution of species, the way organisms adapt to thermal variation is important and deserves a closer look. The results presented in this thesis contribute to the general knowledge of the mechanisms of insect adaptations to environmental variation with the butterfly *B. dorothea* as a model species.

The surveys conducted in different localities in Cameroon revealed the presence of *B. dorothea* in low and mid-altitude moist forests, but not in high altitude and savannas. Furthermore, this study has shown, using selected life history and morphological features of *B. dorothea*, that at 25ºC there is no protandry, but adult males and females show consistent differences which are important since the members on the "*dorothea*-complex" (namely *B. dorothea, B. moyses* and *B. jefferyi*) can be distinguished only with few wing characters. These findings have considerable implications for the systematics and identification of members of the "*dorothea*-complex".

The investigation on seasonal variation in wing morphology across habitats in *B. dorothea* by comparing the wing morphology of wild individuals collected during wet and dry seasons in four localities during two consecutive years has showed that *B. dorothea* exhibits seasonal polyphenism in some of its wing pattern characters and that the degree of plasticity is a function of habitat type. Populations from ecotone habitats (Ako and Ndikiniméki) tend to be more variable over the wet and dry seasons relative to their forest (Mbalmayo and Somalomo) counterparts. This seasonal polyphenism is likely linked to environmental cues such as climate or vegetation, known to vary considerably in ecotone habitats, and driven by selective forces such climate and host plant changes over time. The study here demonstrates the importance of field-based study of butterfly morphology for understanding trait and environment relationships.

The study further investigates the geographic variation for seasonal plasticity in *B. dorothea* by comparing thermal reaction norms for wing pattern and several life history traits of the four populations with the one important question: is there evidence for local adaptation to the specific

climate of each locality, or whether essentially the same plasticity response to developmental temperature covers a broader range of climates? In the light of climate change this question is relevant because, when predicting species' responses to climate change, it is important to understand to what extent phenotypic plasticity allows organisms to cope with changing temperatures. The more specialized is an organism in its phenotypic plasticity response to temperature, the less likely it will successfully cope with changing climatic conditions.

To compare the seasonal plasticity response to developmental temperature between the populations, the reaction norms for wing pattern, development time, and adult size, at three different developmental temperatures (22, 26 and 30ºC) was measured. These temperatures spanned the range of average temperatures experienced by the butterflies in the field. Interestingly, the four *B. dorothea* populations included in this study could be differentiated based on their wing pattern, larval development time and pupal weight, suggesting a potential adaptation of these populations to their local climates.

The response to heat stress revealed important differences among populations with those from ecotone habitats being more tolerant than their forest counterparts. With knowledge of the current mean and occasional peak high temperatures in different parts of the distribution on *B. dorothea* and its thermal limits, these data provide a basis by which the potential harmful effects of climate warming can have on insects' population in different habitat types in Cameroon and elsewhere. In a broader perspective, the results may also add to our understanding of whether and how species may adapt to climate change. It is important to highlight that this investigation is likely the first in Cameroon using a butterfly species as a model system to understand how insects respond to different environmental conditions.

Recommendations

This research demonstrated that populations of *B. dorothea* from different localities react differently to environmental variation in the field and under controlled laboratory conditions. The intensification of recent and future climate change will likely be a serious challenge for tropical ectotherms species. Recommendations based on the results of this thesis can be as follow:

- reduce green gases emissions which are accelerators of climate variations;
- avoid habitat fragmentation that can lead to local climate modification and hence impact on insect's physiology;

- accentuate the development of specific landscape management in Cameroon and neighboring countries to address a regional response to climate change;

Perspectives

This study is one of the few, if not the first, of its kind that assessed the ability of and insect species to adapt to current global changes. It laid the groundwork for future research. In a follow-up of our results, it would be valuable to investigate in more detail the importance of adult acclimation in coping with climatic variation for the labile life history traits, and to determine the extent to which this form of plasticity can buffer the variation predicted as a result of climate change. As such, to complete the results obtained in the framework of this thesis, it will be necessary:

- to conduct a comparative study between the different populations of *B. dorothea* occurring in distinct habitats with the aim to explore the effects of climatic variations between seasons on physiological aspects such as reproductive activities, variation of fat contents, variation in juvenile hormones, the potential effects of the endosymbiont *Wolbachia* which is known to modify various physiological aspects of insects;
- to explore the genetic variation in those population using the advanced method of the single nucleotide polymorphism (SNPs) to assess their potential role in buffering the harmful effects of heat;
- to assess the role of developmental plasticity and the implication of heat shock proteins in the response of cold and heat stresses;
- to assess the potential genetic implication in habitat-specific plasticity in *B. dorothea*.

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1. **Dongmo Kenfak M. A.,** Bonebrake T. C., Fomena A. and Hanna R. (2017). Life history notes on *Bicyclus dorothea* Cramer, 1779 (Nymphalidae: Satyrinae) in Cameroon. *Tropical Lepidoptera Research* 27(1): 28-32.

2. **Dongmo Kenfak M. A.,** Bonebrake T. C., Hanna R. and Fomena A. (2018). Seasonal Polyphenism in *Bicyclus dorothea* (Lepidoptera: Nymphalidae) Across Different Habitats in Cameroon. *Environmental entomology*, 47(6): 1601-1608.

Life history notes on *Bicyclus dorothea* Cramer (Nymphalidae: Satyrinae) in Cameroon

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Abstract: *Bicyclus dorothea dorothea* (Cramer, 1779) is a widely distributed butterfly inhabiting much of the northern part of the tropical African rainforest. The biology of the species has not been well studied despite it being relatively common throughout its distribution. In this study, we report on the life history of *B. d. dorothea* following three years of research on the species in Cameroon. We describe the life cycle of the species and report on key life history aspects such as distribution, habitat, reproduction, and host plant relationships.

INTRODUCTION

The genus *Bicyclus* (Kirby, 1871) constitutes a group of over 100 species and several subspecies found exclusively on the African continent (Aduse-Poku *et al.*, 2017). *Bicyclus* species tend to be common in the understory of a variety of forest types, although a fair number of species inhabit savannah ecosystems. Members of the genera *Bicyclus* and *Hallelesis* Condamin, 1961 were first considered to belong to the genus *Mycalesis* before Condamin in 1961 separated them into two distinct genera. These two genera shared a common Asian ancestor before diverging some 25 million years ago (Aduse-Poku *et al.*, 2015). Condamin's (1973) monograph classified the then-known 77 species into 29 species groups based on their morphology. Later work by Aduse-Poku *et al.* (2017) updated the systematics of the genus and arranged the now 103 species into sixteen revised species groups largely following Condamin's earlier work. Despite the genus now being fairly well resolved systematically, identification of many species remains challenging. Many species are very similar in appearance to one another, and, in such cases, examination of male androconial brushes located beneath the hindwings are often used for identification. In some cases, females cannot be identified using wing morphology alone.

Bicyclus dorothea dorothea (Cramer, 1779), or the Light Bush Brown, might be one the most common forest satyrines in tropical African rainforests (Larsen, 2005). In contrast to other satyrines in the region, *B. d. dorothea* is paler in color and relatively easy to identify from other satyrines. A recent phylogeny of *Bicyclus* demonstrated that *B. dorothea* can be grouped into a "*dorothea*-complex" consisting of *B. dorothea, B. moyses* Condamin & Fox, 1963 and *B. jeyfferyi* Fox, 1963 based on significant morphological and genetic similarities between the three species (Aduse-Poku *et al.*, 2017). In any case, little is known about the biology or ecology of *B. dorothea*. Apart from a few notes made on its immature stages in a comparison to other immature stages of *Bicyclus* and *Hallelesis* (Sourakov & Emmel, 1997) and some rearing experiments at Lamto, Ivory Coast (Vuattoux, 1994), little has been published with regards to its biology or natural history.

In this paper, we describe life history features of *B. d. dorothea* in Cameroon, with the ultimate goal of providing a background for further study of this species and other African satyrines. We report here on field observations and rearing records from research on the species between 2014 and 2016.

RESULTS AND DISCUSSION

Distribution

Bicyclus d. dorothea is a largely tropical species found in the whole west African forest zone. Its distribution extends to southern Cameroon, the northern part of the republic of Congo, Democratic Republic of Congo and Central African Republic (Aduse-Poku *et al.*, 2017). While Larsen (2005) and van de Weghe (2010) stated its presence in northern Angola, recent studies by Aduse-Poku *et al.* (2017) show that it is *B. moyses* which is present there. In addition, none of these studies noted its presence in Gabon, but it has recently been recorded at the Minkébé National Park, northern Gabon (van de Weghe 2016, pers. comm). The subspecies *B. d. concolor* Condamin & Fox, 1964 inhabits São Tomé, Principe, and Bioko islands (Larsen, 2005). Among species of the "*dorothea*-complex", *B. moyses* has a sympatric distribution with *B. d. dorothea* in southern Cameroon, northern Democratic Republic of Congo and the Republic of Congo, while in Angola, southern Democratic Republic of Congo, Republic of Congo, Gabon and Angola, only *B. moyses* is present. Of the remaining species of the complex, *B. jeyfferyi* is mostly found in eastern Africa. *Bicyclus d. dorothea* seems to be distributed in low to mid elevation habitats. Out of 28 localities sampled in Cameroon (Fig. 1), only one individual has been caught above 1000 m above sea level (1032 m on Mount Cameroon).

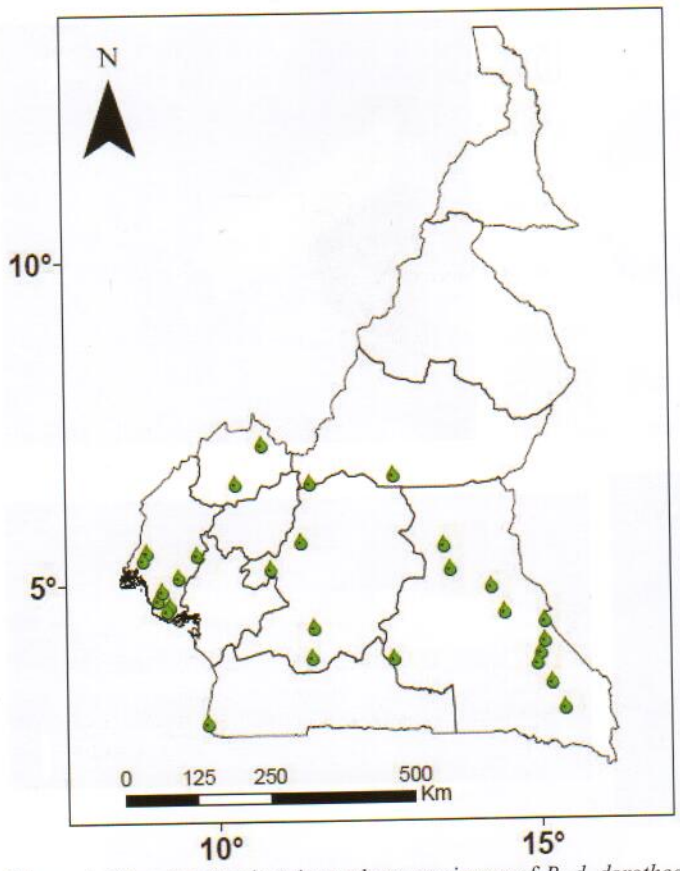

Figure 1. Map showing locations where specimens of *B. d. dorothea* have been collected in Cameroon from 2014 to 2016.

Habitat

Bicyclus d. dorothea inhabits a wide variety of forest types and appears particularly to favor degraded or disturbed forests habitats (Larsen, 2005). It can also be found in forest-savannah ecotones where population sizes appear relatively low compared to forest habitats. From our observations in the field, the species is rarely encountered in dense forest understory habitats, although occasionally a female will fly away from a threat into the understory. Generally, *B. d. dorothea* is found in degraded forests where colonies establish in clearings and road tracks receiving sun and where larval host plants and adult food resources are abundant. Agricultural lands harboring canopy structures like palm farms, fruit trees and, cacao farms are also optimal habitats for the species, probably because these microhabitats provide abundant food resources such as the decaying fruits on which adults feed.

Flight activity starts as soon as the sun has risen, but under intense sunshine conditions adults will retreat into shade. Males are abundant throughout the day while female numbers increase in the afternoon (from 14:00 to 17:00) when they display mating and oviposition behavior. *Bicyclus dorothea* individuals usually fly alone, although it is common to see contest behaviors in males.

Immature stages

Eggs

Bicyclus dorothea eggs are white in color and similar to other *Bicyclus* species (Sourakov & Emmel, 1997). Eggs are laid singly or in patches of two to ten on the underside of host plant leaves (Fig. 2a) and typically take three to five days to hatch under natural conditions. One day before hatching, the upper pole of the egg will turn black (Fig. 2b), representing the dark head capsule of the developing larva.

First instar larvae

After the egg maturation, the young caterpillar ecloses by nibbling a portion of the egg's shell. The newly emerged first instar larva has a 3 to 3.5 mm long, cylindrical white body covered by dorso-lateral rows of setae (Fig. 2c). At the posterior end of the body, a pair of backward-pointing setae can be easily observed. The head is dark and bears setae, protuberances, and one pair of short horns. First instar larvae are mobile immediately after eclosion and their body color turns greenish over time. After about three to five days, they molt into the second instar.

Second instar

Molting in *B. dorothea* is consistent with other immature stages of *Bicyclus* species (Sourakov & Emmel, 1997). Larvae will outgrow their head capsule, with the cuticle of the body remaining intact. The second instar larva of *B. dorothea* is characterized by a brownish head capsule with a well distinguished pair of horns; the body is greenish with more pronounced setae relative to the first instar (Fig. 2d). This instar lasts four to six days before reaching the next stage.

Later larval instars

The next instars (third, fourth and fifth) differ from the previous essentially in terms of body length and head capsule colors (Fig. 2e-g). The body color is quite uniform and there is an increase of the body length as they grow. The third instar has a dark cephalic capsule with green striations in front. Horns are distinguishable and this stage takes about four to six days to complete. The next two molts bring the caterpillar to fourth and fifth instars.

Pre-pupal and pupal stages

At the pre-pupal stage the body of the caterpillar gradually shrinks in length and the caterpillar finds a spot on the underside of a leaf blade where it spins a silk pad (Fig. 2h). It then brings its head towards the anal end to form a pre-pupa. This stage lasts one day at ambient temperature but can reach up to three days when reared at temperatures lower than 20°C.

The pupa of *B. dorothea* is green in color and approximately cylindrical (Fig. 2i). Pupation takes place as follows: once the pre-pupa is matured, a slot opens on the head capsule and the hanging caterpillar makes uncoordinated movements to facilitate shedding of the body cuticle toward the anal end. In most cases, the shed cuticle is removed from the fresh, soft pupa which hardens a few hours later. This stage lasts six to eight days under ambient conditions, and the pupa darkens in coloration one day before the imago's emergence (Fig. 2j).

Adult variation and identification

One of the most visible wing features in *Bicyclus* is the presence of eyespots. Many *Bicyclus* species display strong phenotypic variation of these traits over seasons (Brakefield & Reistma, 1991; Roskam & Brakefield, 1999; Brakefield *et al.*, 1998). *B. dorothea* adults have among the lightest colored eyespots of the group, although the species is very similar to

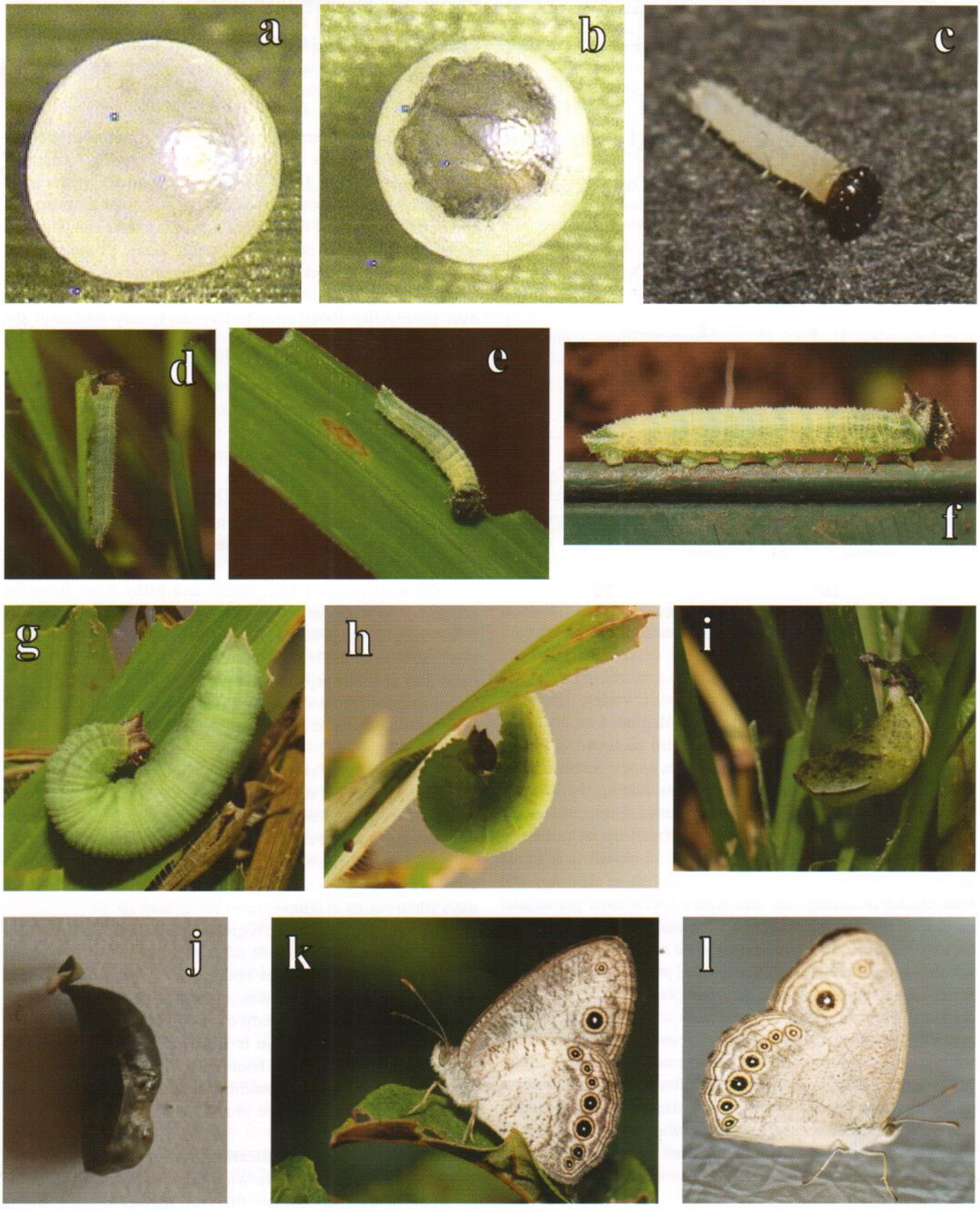

Figure 2. Life cycle of *B. d. dorothea*: (a) newly laid egg; (b) egg on a lawn leaf with the head of the first instar caterpillar visible through the egg shell; (c) first larval instar; (d) second larval instar; (e) third larval instar; (f) fourth larval instar; (g) fifth larval instar; (h) prepupa; (i) early pupa; (j) pupa a few hours before the emergence of the adult; (k) ventral view of an adult male (Photographed by Oskar Brattström in Liberia); (l) ventral view of an adult female.

other members of the "*dorothea*-complex", namely *Bicyclus moyses* and *B. jefferyi*. There is low genetic divergence among these species, but there are consistent differences in wing pattern (Aduse-Poku *et al.*, 2017). *B. dorothea* possess two eyespots on the forewing and seven on the hind wing, although sometimes individuals can display three eyespots on the forewing and eight on the hind wing. The mean wing span is about 40 mm; in males, the discal area of the forewings and the basal half of the hind wings are light grey while the rest of the wing is darker (Fig. 2k, Fig. 3a). The underside of the wing is gray-beige with the eyespots not visible. The male's hind wing bears two androconial brushes which are absent in females; this is one of the main features that can be used to differentiate *B. dorothea*, *B. moyses* and *B. jefferyi*. In fact, males of all three species have two androconial brushes in cell Rs and CuA2 of the hind wings, but in *B. dorothea* the brush is light brown to yellow and weakly developed in cell Rs while in *B. moyses* the brush is darker, and it is almost black in *B. jefferyi* (Aduse-Poku *et al.*, 2017). The female wings are uniformly light grey in *B. dorothea* (Fig. 2l, Fig. 3b).

Laval host plants and adult feeding

Larvae of the genus *Bicyclus* feed primarily on grasses (Poaceae). A semi-artificial diet made of bean flour has been developed and has been used to successfully rear immature stages of *B. anynana*, *B. ena* and *B. safitza* (Holloway *et al.*, 1991). Many species in the family Poaceae are used as host plants by *B. dorothea*. Larvae have been reared on the genera *Oplismenus*, *Paspalum* and *Axonopus* at Lamto, Ivory Coast (Vuattoux, 1994). In our research, we reared *B. dorothea* on millet, *Pinnesetum glaucum*, and *Axonopus*, but all trials with maize (*Zea mays*) were unsuccessful, in contrast to *B. anynana* where maize may be used for rearing (Kooi, 1992).

Whilst a majority of adult butterflies obtain carbohydrates by feeding on nectar (Bonebrake *et al.*, 2010), satyrine species usually feed on decaying fruits (Boggs, 1997a,b). *Bicyclus dorothea* feeds on fallen fruits found in the forest such as guava, overripe banana, umbrella fruit tree, mangoes and many other species. Although we have observed a few individuals exhibiting mud-puddling behaviour, we have never seen the species foraging on dung or on nectar.

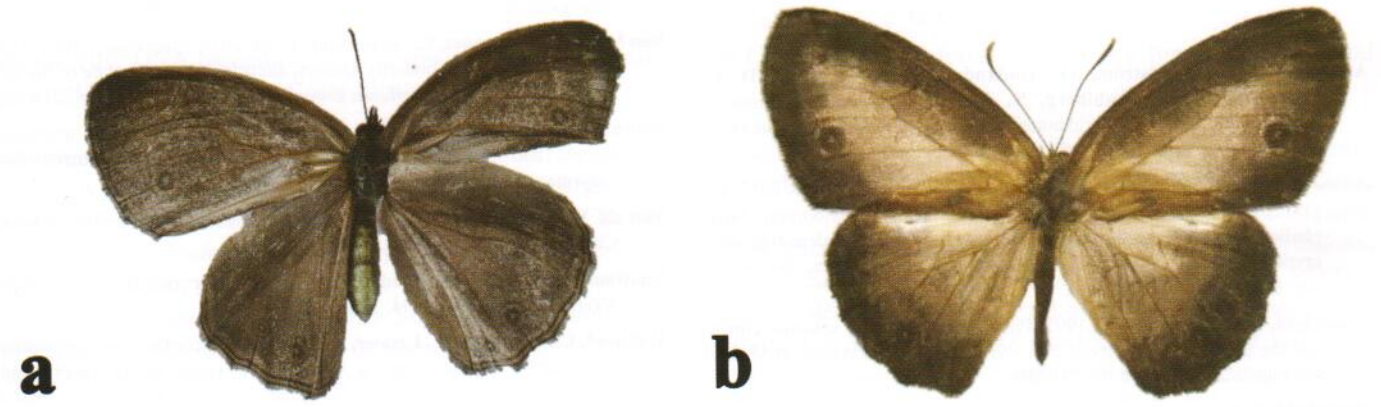

Figure 3. Dorsal view of (a) an adult male and (b) an adult female of *B. dorothea*.

Mating, reproduction and adult life span

Mating, reproduction, and adult life span have been well studied in *B. anynana* (Pijpe, 2007; Geister *et al.*, 2008), and this research serves as a useful comparison with *B. dorothea*. These studies have highlighted the role of experimental set up (Brakefield *et al.*, 2009), dorsal eyespot and wing sizes (Breuker & Brakefield, 2002), sex pheromone production, sexual conflicts in males (Wiklund *et al.*, 2001), sexual selection and the problem of protandry in reproduction (Zwaan *et al.*, 2008; San Martin *et al.*, 2011). In *B. anynana*, males and females are generally pooled together following eclosion in hanging cages for mating purposes. Females tend to be ready to mate after two or three days, after which they lay eggs during the two following weeks (Brakefield *et al.*, 2009). Reproduction in *B. dorothea* appears to be similar to *B. anynana*, although some differences are apparent; for example, *B. dorothea* females generally do not mate for approximately 10 days after eclosion. Moreover, mating seems to be more challenging in *B. dorothea* than in *B. anynana* and we had some difficulty producing viable eggs from lab-mated females. This may be due to the fact that *B. anynana* used in many laboratories in Europe has been selected for many generations in the lab to be quick to mate. Almost all other species tested have a relatively long pre-oviposition period. Another possible explanation is that *B. anynana* exploits a short-lived rainy season, so mating quickly is advantageous, while forest-associated species can breed throughout the year and can afford to be more selective in mate choice and timing.

Despite some difficulties in initiating successful mating, we did eventually observe frequent mating pairs in the lab and also in the field. In the lab, we also frequently observed females laying unfertilized eggs, likely as a consequence of unsuccessful mating. As observed in many insects, female *B. dorothea* lay most of their eggs in the first two weeks following the first oviposition event. Life span, like other traits, varies with environmental conditions, but we observed that at 25°C *B. dorothea* adults can survive up to 60 days in the lab.

CONCLUSION

Research on *B. anynana* has developed the species into a model organism for topics such as development, ecology and evolution. In recent years, additional research on other *Bicyclus* species has provided an opportunity for powerful comparative approaches (Brakefield, 2010). In this paper, we have provided notes on *B. dorothea* life history which we hope will aid further investigation of this and other *Bicyclus* species. Such research is particularly important in Cameroon, where the ecological impacts of anthropogenic change are significant, but remain poorly understood (Lawton *et al.*, 1998).

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Research

Seasonal Polyphenism in *Bicyclus dorothea* (Lepidoptera: Nymphalidae) Across Different Habitats in Cameroon

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Abstract

Many organisms exhibit changes in phenotypic traits as a response to seasonal environmental variation. We investigated the role of habitat in generating seasonal polyphenism in different populations of the light bush brown butterfly *Bicyclus dorothea* (Cramer, 1779) (Lepidoptera: Nymphalidae) in Cameroon. Butterflies were caught during the wet and dry seasons across four localities representing two distinct habitats, namely forest and ecotone (forest–savanna transition zone) over a 2-yr period (2015–2016). We found distinct variation in the wing pattern characteristics of butterflies in response to seasonality and habitat. Specifically we observed that: 1) all wing characters are not seasonally plastic in *B. dorothea*; 2) populations from ecotone tend to be more variable, with individuals exhibiting wings with large spots during the wet season and very reduced spots in the dry season while in forest populations, individuals exhibit wings with large spots during the wet season, but in the dry season, spots are not as greatly reduced as their ecotone counterparts; 3) this polyphenism in *B. dorothea* alternated consistently during the wet and dry seasons over the 2 yr of sampling. *Bicyclus* species have become a textbook example of seasonal polyphenism while this study extends this model system to the unique forest–ecotone gradient of Central Africa and demonstrates the complexity of seasonal forms in different habitats.

Key words: seasonal polyphenism, *Bicyclus dorothea*, ecotone, forest

Polyphenism is a phenomenon characterized by the production of two or more distinct phenotypes from a single genotype (Shapiro 1976). This phenomenon is common in many insect species who exhibit this feature as a response to variation in an environmental cue (Simpson et al. 2011). In a seasonal environment, polyphenism will be the result of contrasting but predictable cues, leading to different morphologies, life history traits or behaviors expressed in each season and allowing species to persist in a given environment (David et al. 1997, Oostra et al. 2011). These environmental cues can be temporal, spatial, biotic or abiotic (Whitman and Agrawal 2009). Polyphenism is thought to be an adaptive response and potentially a determining factor in the evolutionary success of insects (Simpson et al. 2011).

In recent decades, multiple aspects of polyphenism in insects have been studied (Capy 1993, David et al. 1997, Ribeiro and Freitas 2011). Though attention has been paid to a range of Lepidoptera species such as *Melanitis leda* (Brakefield and Larsen 1984, Brakefield 1987), *Manduca sexta* (Kingsolver et al. 2009), and *Automeris io* (Sourakov 2014), species belonging to the subtribe Mycalesina and the genus *Bicyclus* (Kirby, 1871) represent the best-studied taxa with respect to phenotypic plasticity in the field (Brakefield and Reitsma 1991, Windig et al. 1994, Brakefield and Frankino 2009, Brakefield and Zwaan, 2011). Studies in phenotypic plasticity have used many *Bicyclus* species such as *Bicyclus anynana* (De Jong et al. 2010), *B. cottrelli*, *B. safitza*, *B. ena*, *B. vulgaris* and *B. vansoni* (Windig et al. 1994, Roskam and Brakefield 1996), and *B. sanaos* (Oostra et al. 2014, van Bergen et al. 2017). Studies conducted in Malawi have shown that during the dry season (characterized by scarce nutritive resources and a drop of monthly mean temperatures) adult *B. anynana* typically express wings with small cryptic eyespots (Brakefield and Reistma 1991, Brakefield 1997). Moreover, apart from wing patterns, many other life history traits are also affected such as reproductive diapause, fat content, longevity, weight, development time of immature stages, and predator avoidance (Kooi et al. 1997, Zwaan et al. 2001, Zijlstra et al. 2002, Lyytinen et al. 2004, Westerman and Monteiro 2016). During the wet season, resources are abundant and adult butterflies display wings with prominent and concentric eyespots along the distal margin. These favorable conditions allow butterflies to develop faster and produce two or three generations before the onset of the dry season. This polyphenism in *B. anynana* wing patterns appears to be mainly induced by temperature variation over wet (high temperature) and dry (low temperature) seasons in their natural habitat in Malawi (Brakefield et al. 2007).

In fact, *B. anynana* cohorts reared at 27°C in the laboratory exhibit wet season forms with prominent eyespots while those reared at temperatures ranging between 17 and 20°C exhibit dry season forms with cryptic eyespots (De Jong et al. 2010).

Central Africa is dominated by Congolese forest habitats that cover most of equatorial Africa. However, there is also considerable habitat variation throughout the biome where forests meet with other ecological communities, ecosystems or ecological regions at their boundary forming a community mosaic termed the 'ecotone' (McArthur and Sanderson 1999, Kark and Van Rensburg 2006). Ecotones occur at multiple spatial scales and range from natural boundaries to human-generated ecotones. These transition zones have also been highlighted as high speciation centers and hence many researchers have noted their importance for long-term conservation (Smith et al. 1997, Walker et al. 2003, Kark 2013, Senft 2009). In Cameroon, forest–savanna ecotones are present and represent an opportunity to address questions regarding habitat influence on seasonal polyphenism. Forest and ecotone habitats may present fluctuations in many climate components such as annual rainfall, monthly mean temperature, and vegetation composition which may exert influence on phenotypic plasticity in insects.

This study investigates seasonal variation in the morphological traits of *Bicyclus dorothea* (Cramer, 1779) (Lepidoptera: Nymphalidae), congener of *B. anynana*, across different habitats (forest and ecotone) of Cameroon. Physiognomic and climatic variation between forest and ecotone habitats may drive divergence in morphological responses to seasonality in *B. dorothea*. In this context, we expected populations from climatically less variable forest habitats to show lower variation in morphological features compared to populations from the more variable ecotone habitats. In this study, we first determined 1) which morphological features are seasonally plastic in *B. dorothea* by measuring wing features on individuals caught in each habitat over time across a year. We then examined 2) the extent to which forest and ecotone habitats differ from one another in structuring morphological variation of *B. dorothea* across seasons.

Material and Methods

Species

B. dorothea is a tropical fruit-feeding butterfly found in West and Central Africa (Condamin 1973). It is found in the whole West African forest zone. Its distribution extends to southern Cameroon, the northern part of the Republic of Congo, Democratic Republic of Congo, Central African Republic and reaches the Semuliki forest at the border with Uganda (Aduse-Poku et al. 2017). Where it occurs, *B. dorothea* seems to prefer low- to mid-altitude habitats. In Cameroon, *B. dorothea* is found widely in mid-altitude forests and across many forest–savanna ecotone transition areas. It is uncommon or absent in highland and dry savanna habitats (Dongmo et al. 2017). Up to now, no studies have investigated the dispersal ability or behavior of *B. dorothea* adults. However, from our observations in the field, adults appear to be relatively sedentary and are generally found reliably and restricted locally to sites where larval host plants and adult food resources are abundant.

Study Sites and Sample Collections

Cameroon is a central African country with highly variable topography and associated vegetation and climatic parameters across large altitudinal gradients. The south of the country consists of tropical rainforest while the north and far north is made up mainly of lowland savanna. We used four sites in Cameroon to determine the relationship of vegetation and climate on polyphenism in *B. dorothea*—two in the tropical rain forest, Mbalmayo (N 3.388, E 11.47, 768 masl) characterized by a degraded forest and Somalomo (N 3.37405, E 12.7332, 638 masl), a primary forest in the Dja wildlife reserve. The two other sites, a woodland forest–savanna ecotone at Ako (N 6.68783, E 10.70687, 706 masl) and a forest–savanna ecotone at Ndikiniméki (N 4.76986, E 12.7332, 812 masl) were also sampled, representing ecotone habitat (Fig. 1). We used Thermochron iButton data loggers (model: DS1922) in each site to record ambient temperature. Data loggers were suspended at 2 m from the ground and protected from direct sun. Rainfall data were obtained from the Mbalmayo weather station and approximated for the remaining sites—Bafia (50 Km from Ndikiniméki),

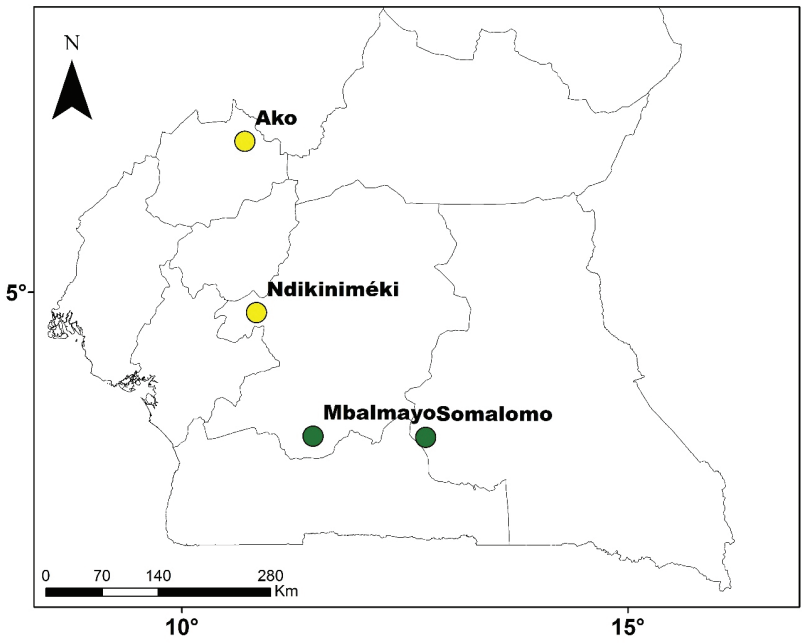

Fig. 1. Map showing sampling sites in Cameroon. Yellow circles represent ecotone sites while green circles represent forest.

Akonolinga (41 Km from Somalomo), and Bambui (80 Km from Ako). In all study sites, seasonal timing is similar with the onset of the dry season in mid-November extending to early March. However, there are differences in the amount and variation in temperature and rainfall (Fig. 3a–d). The ecotone sites (Ako and Ndikiniméki) have a unimodal rainfall pattern with reduced precipitation from November to February (Fig. 3a and b) while in the forest sites, the rainfall pattern is bimodal with relatively high precipitation during the dry season (November to February) compared to ecotone locations (Fig. 3c and d).

At each site, individual butterflies were caught using sweep nets in addition to over-ripe banana-baited traps placed in road tracks or clearings in the forest, a preferred microhabitat of *B. dorothea* (Larsen 2005). Sampling was conducted two or three times during the dry (trapping from January to March) and wet (trapping from June to August) seasons in both 2015 and 2016 at each locality. Butterflies caught were brought to the entomology laboratory of the International Institute of Tropical Agriculture (IITA-Yaoundé, Cameroon) and kept in a room maintained at 26°C, 75% RH and 12:12 (L:D) h photoperiod and used for rearing study (for a related study not described here). After their death, wild-caught individuals were stored in small glassine envelopes for further morphological analysis.

Morphological Measurement

The wing spots of *B. dorothea* are similar in structure and position to most *Bicyclus* species; however, only ventral spots are present and dorsal spots are absent in this species. There are nine spots in total, two on the forewings and seven on the hindwings, though sometimes one can find three spots on the forewing and eight on the hindwing of some individuals. Eyespots are approximately circular in shape and made of numerous rings: a white ring in the center of the eyespot, a black disc, a cream yellow disc and an outer gold disc (Brakefield and French 1997). Prior to measurement, fore and hindwings were separated from the body of dead butterflies. Thirteen wing pattern characters (Fig. 2) were measured. Since the spots are not perfectly circular, all measurements were done parallel to the vein of the wing. Moreover, measurements were done only on the left fore and hindwings. The right wings were used in cases where the left wings exhibited significant damage. To measure the diameter of the eyespot, the fore and hindwings were placed between two glass slides under a stereomicroscope fitted with a micrometer eyepiece at 6× magnification. Wing length, from the thorax to the apex and the width of the fore and hindwings were measured using a caliper (0.1 mm accuracy).

Statistical Analysis

To analyze the morphological variation in wing characters, a principal component analysis (PCA) was performed on data of each sex to reduce the wing pattern variability for forest and ecotone habitats. The first two components were used for further analysis (see results for further details). We then applied nested analysis of variance (ANOVA) models on the two retained principal components with season, habitat, and sex as fixed factors, and sampling sites nested in habitat. We constructed models with interaction terms between season and habitat, and with sites nested in habitat. Visual inspection of residual plots of each model did not reveal any obvious deviations from homoscedasticity or normality. *P*-values were obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. All statistical operations were performed with R v 3.4.0 software (Team, R. C. 2017).

Fig. 2. Characters measured by the stereo microscope and caliper. Shown are one fore and one hind wing of a *B. dorothea* male. 1 = length of the fore wing; 2 = width of the fore wing; 3 = diameter of the first eyespot of the fore wing; 4 = diameter of the second eyespot of the fore wing; 5 = length of the hind wing; 6 = width of the hind wing; 7 = diameter of the first eyespot of the hind wing; 8 = diameter of the second eyespot of the hind wing; 9 = diameter of the third eyespot of the hind wing; 10 = diameter of the fourth eyespot of the hind wing; 11 = diameter of the fifth eyespot of the hind wing; 12 = diameter of the sixth eyespot of the hind wing; 13 = diameter of the seventh eyespot of the hind wing.

Table 1. Number of butterflies captured during 2 yr in each site

Sampling sites	Habitat	Sex	Seasons				Total
			Wet 2015	Dry 2015	Wet 2016	Dry 2016	
Ako	Ecotone	Males	83	69	44	38	369
		Females	31	26	50	28	
Ndikiniméki		Males	72	69	54	43	382
		Females	50	27	39	28	
Mbalmayo	Forest	Males	74	69	67	58	434
		Females	54	34	56	22	
Somalomo		Males	30	93	36	28	313
		Females	34	36	35	21	

Results

We recorded a total of 1,498 butterflies during 2 yr of sampling in four sites representing two distinct habitats (Table 1). PCA performed on our data recorded 74.4% of the total variation of the first two principal components (PC1 and PC2) in males and 75.5% in females. The diameter of wing spots loaded heavily on PC1 while the length and the width of the fore and hindwings had the highest loadings on PC2 for both males and females (Table 2). PC1 and PC2 varied across seasons and habitat (Fig. 4). The diameter of the eyespots (PC1) are the main wing pattern characters varying across season and habitat while PC2 showed less variation, suggesting that the length and the width of the wings are not seasonally plastic and are less affected by habitat (Fig. 5). Within each habitat, PC1 fluctuated seasonally and simultaneously, but the pattern of variation was not identical across habitats. The diameter of ocelli in the ecotone population varied drastically from one season to another while in forest populations, a slight variation of this trait

Table 2. Component weights and eigenvalues for the principal component analysis with data of all habitat and seasons pooled together in males and females

Traits	Males		Females	
	PC1	PC2	PC1	PC2
Length of the forewing	0.099	0.561	−0.150	0.506
Length of the hind wing	0.125	0.549	−0.153	0.481
Width of the forewing	−0.019	0.054	−0.163	0.406
Width of the hind wing	0.138	0.565	−0.176	0.485
Diameter first eyespot forewing	0.333	−0.097	−0.325	−0.128
Diameter second eyespot forewing	0.346	−0.053	−0.329	−0.116
Diameter first eyespot hind wing	0.293	−0.082	−0.287	−0.113
Diameter second eyespot hind wing	0.345	−0.106	−0.322	−0.135
Diameter third eyespot hind wing	0.351	−0.093	−0.329	−0.139
Diameter fourth eyespot hind wing	0.346	−0.093	−0.335	−0.121
Diameter fifth eyespot hind wing	0.356	−0.064	−0.339	−0.105
Diameter sixth eyespot hind wing	0.338	−0.047	−0.314	−0.044
Diameter seventh eyespot hind wing	0.181	0.078	−0.243	−0.038
Eigenvalue	55.56%	18.85%	54.17%	21.36%

Eigenvalues are expressed as percentage of total variance accounted for by the first principal component and second principal component.

Table 3. Statistical results of the nested ANOVA models for the relationships between the principal component 1 and 2 and environmental factors

Response trait	Factors	df	Mean sq.	*F* value	*P* value
PC1	Sex	1	0	0.003	<0.001
	Seasons	3	847.0	214.1	<0.001
	Habitat	1	786.6	198.9	<0.001
	Seasons*habitat	3	86.0	21.7	<0.001
	Seasons* habitat/sites	8	102.4	25.9	<0.001
PC2	Sex	1	0.5	0.2	0.623
	Seasons	3	11.8	6.2	<0.001
	Habitat	1	5.9	3.1	0.080
	Seasons*habitat	3	8.9	4.7	0.002
	Seasons*habitat/sites	8	8.3	4.4	<0.001

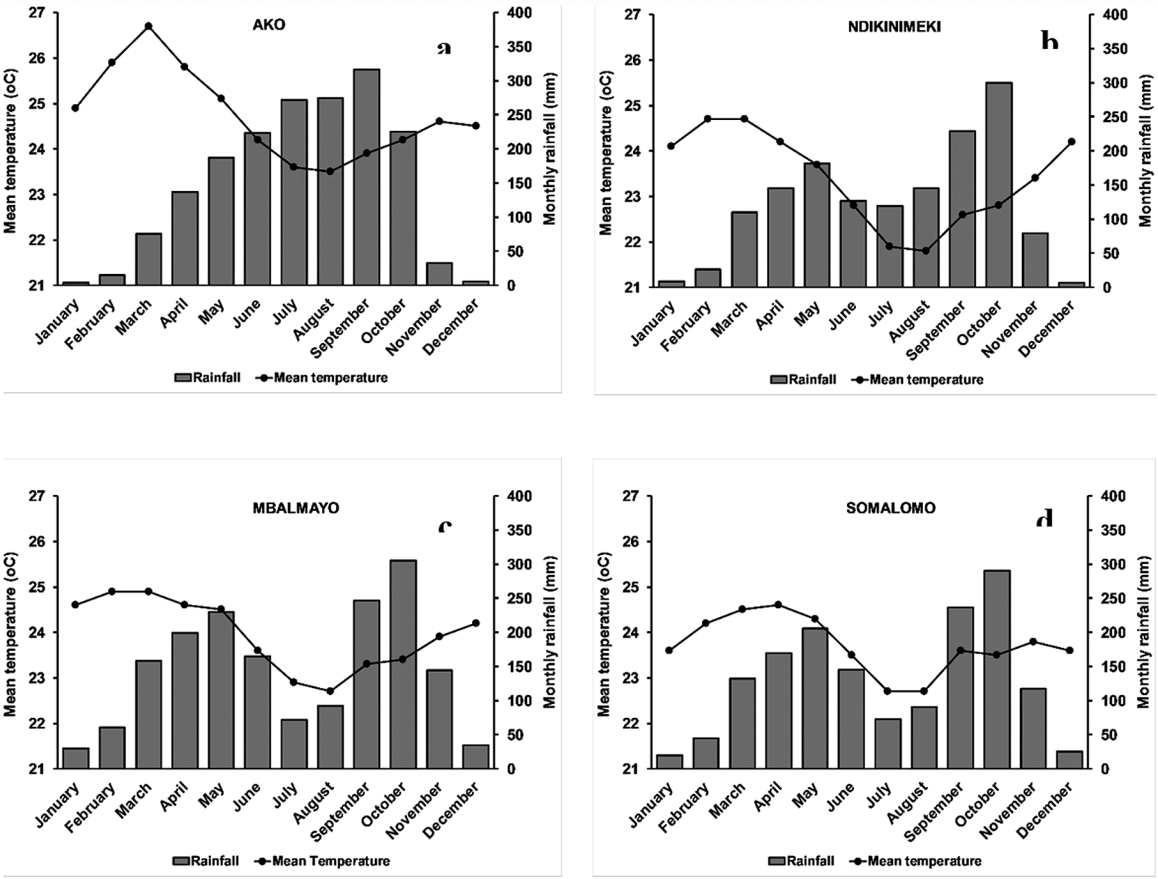

Fig. 3. Temperature and rainfall profiles of each study site: (a) Ako; (b) Ndikinimeki; (c) Mbalmayo; and (d) Somalomo.

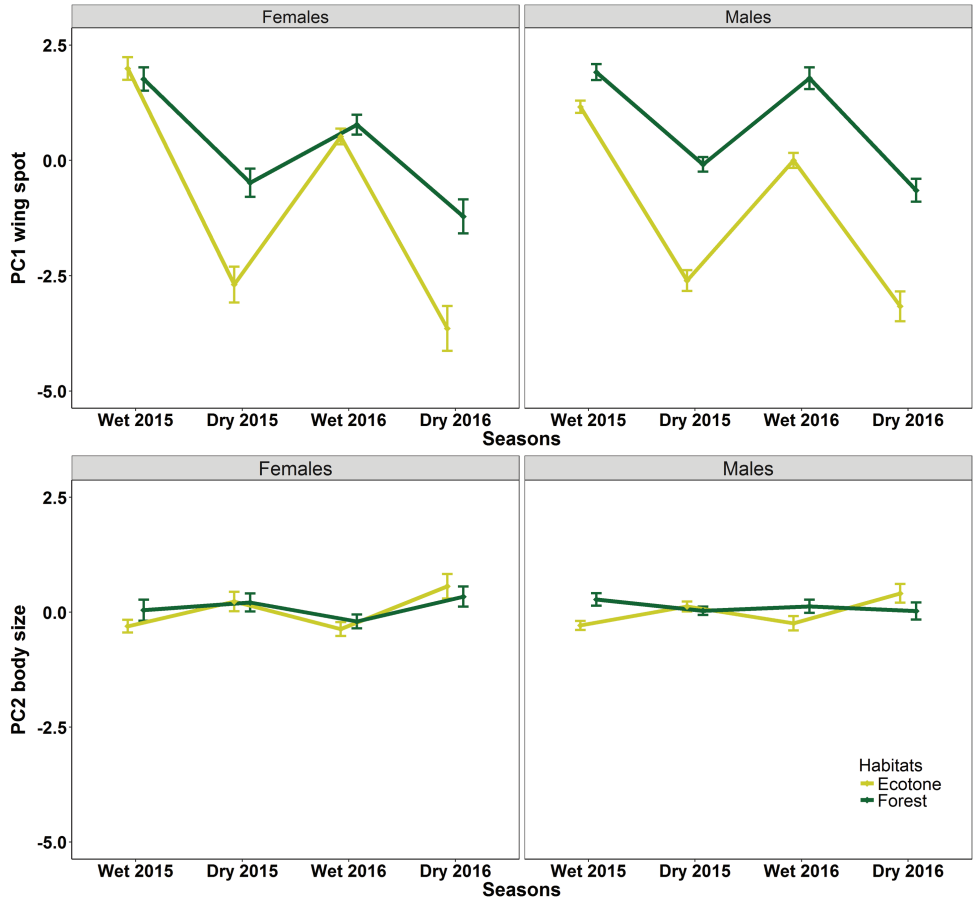

Fig. 4. Seasonal changes in the first two principal components of *Bicyclus dorothea* wing patterns in different populations in Cameroon inhabiting forest and ecotone habitats over the transition from a wet to dry season.

was observed. Nested ANOVA performed using PC1 and PC2 showed a high response for these traits to season, habitat and their interaction though PC2 showed no effect of habitat (Table 3).

Discussion and Conclusions

The results demonstrate that habitat and season have strong effects on wing pattern elements in *B. dorothea*. Among all wing characters studied, the diameter of eyespots was highly sensitive to the effect of habitat, sex, seasons and the interaction between habitat and seasons. All populations examined in this study show a certain degree of plasticity with respect to the diameter of their eyespots. This variation is strongly seasonal and more pronounced in ecotone populations than in their forest counterparts. Generally, this study revealed two important patterns in seasonal polyphenism in *B. dorothea*: 1) a general observation indicating that ecotone populations tend to be more variable than forest populations and 2) change in eyespot size occurs seasonally suggesting an adaptation of these populations to alternating changes in climate annually (Brakefield and Larsen 1984, Brakefield and Reitsma 1991, Brakefield et al. 2007).

Previous studies on seasonal polyphenism in *Bicyclus* butterflies in a single locality in Malawi showed that seasonal forms of these butterflies differ drastically in color and wing pattern elements over the wet and dry seasons (Windig et al. 1994). A similar example was also found in Australia where butterflies belonging to the genus *Mycalesis* Hübner, 1818 exhibit the same pattern of plasticity of their wing elements over seasons (Braby 1994). This plastic response in *Bicyclus* butterflies to wet and dry seasons was shown to be directly related to changes in environmental components (Brakefield and Reitsma 1991, Windig et al. 1994, Brakefield and Zwaan 2011). The role of temperature was highlighted as the main factor inducing these different morphs (Roskam and Brakefield 1996, Brakefield et al. 2007) though biotic factors like food plant quality can also influence the development of wing patterns and hence, can drive plastic responses in the field (Kooi et al. 1996). One of the key climatic characteristics of ecotones is the greater annual variation in environmental variables (Longman and Jenik 1992). In these regions, while rainfall patterns are generally unimodal with large differences between day and night temperatures (Hirota et al. 2010, Ibanez et al. 2013), tropical rainforests are often characterized by a bimodal rainfall pattern, with relatively small differences between day and night temperatures.

Brakefield and Reitsma (1991) highlighted that temperature decreases during the dry season in a unique study area in Malawi was the principal cue leading to the dry season form (with reduced wing spots) of five *Bicyclus* species in the wild. The strong plasticity observed at ecotones during the dry season can be explained by the heterogenous nature of the environment. Ecotones are generally mosaics of forest fragments surrounded by tree-less savanna. Together, vegetation patterns and temperature fluctuations increase environmental heterogeneity at the microhabitat level. In regions with a unimodal rainfall pattern, dry seasons tend to be longer and drier than those of regions with bimodal rainfall patterns (though this also depends on altitude), leading to drier environments in

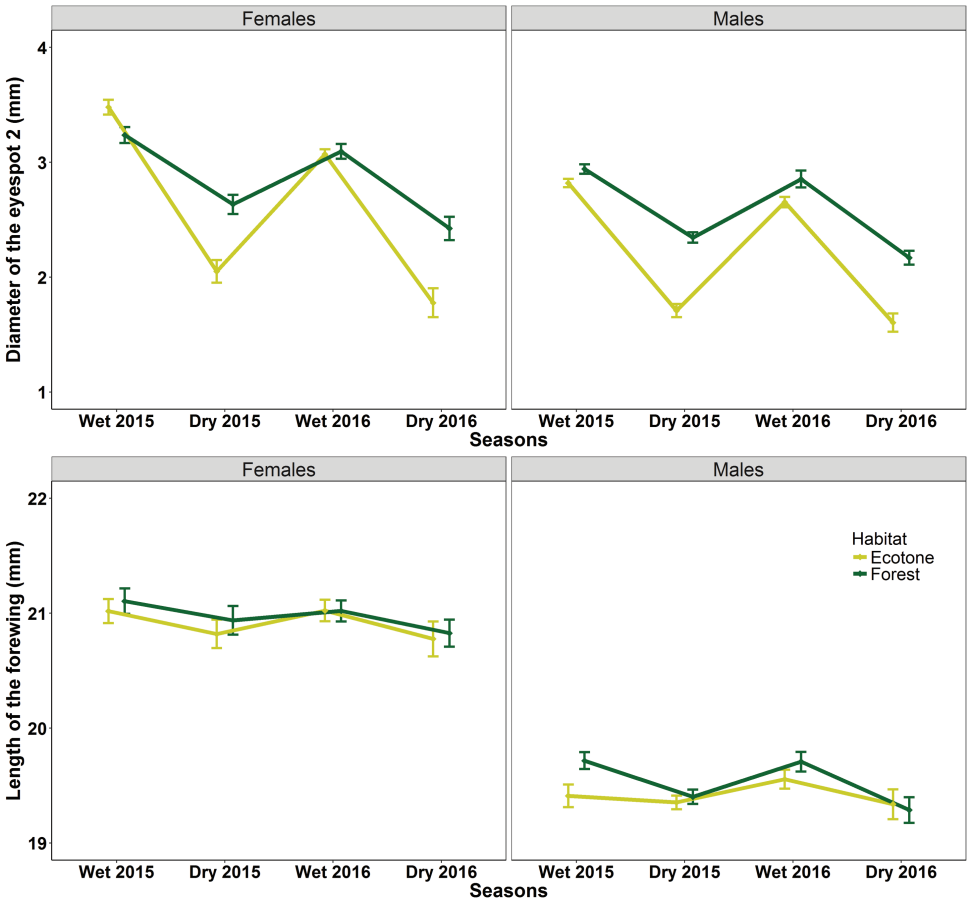

Fig. 5. Seasonal changes in the length of the forewing and the second diameter of the forewing of *B. dorothea* wings in different populations in Cameroon inhabiting forest and ecotone habitats over the transition of the wet to dry seasons.

ecotones during the dry season compared to forest environments (Tsalefac et al. 2003). Hence, while tropical rainforests have almost constant mean monthly temperatures and availability of larval host plant throughout the year, larval host plants are likely only available in the wet season in ecotone habitats where there is considerable fluctuation in mean monthly temperatures over seasons (Chan et al. 2016). Predation very likely plays an additional key role in maintaining wing spot plasticity. In *B. anynana* changes in wing patterns are known to be adaptive in that dry season forms are cryptic while conspicuous eyespots in wet season forms can contribute to deflection of and survival from predator attacks (Lyytinen et al. 2004, Prudic et al. 2015).

It is well known that behavioral responses are among the most immediate responses of animals in general and butterflies in particular to climatic variation (Walther et al. 2002, Parmesan 2006). Two strategies can be used by many satyrine species: 1) adults will cease their reproduction during unfavorable dry seasons and females will start reproducing at the onset of the rainy season (Brakefield and Larsen 1984), or 2) persisting adult females during the dry season will move into an aggregate of moist refugia in the understory where they will sometimes lay their eggs on the few remaining fresh host plants for larval growth (Braby 1995). Larvae from these eggs will then develop in an environment with favorable temperatures and will likely yield adults with reduced spots. The high plasticity observed in ecotone populations suggests adaptation to complex changes (such as lack of larval food plants and predator avoidance) occurring during the dry season—but the mechanisms behind these patterns require further study. Exploring seasonal polyphenism in life history traits such as reproductive diapause, egg fertility, population dynamics of *Bicyclus* species across the ecotone and forest gradient will provide insights into such evolutionary aspects of phenotypic plasticity.

Our study showed that *B. dorothea* exhibits seasonal polyphenism in some of its wing pattern characters and the degree of plasticity is a function of habitat type. Populations from ecotone habitats tend to be more variable over the wet and dry seasons relative to their forest counterparts. This seasonal polyphenism is likely linked to environmental cues such as climate or vegetation, known to vary considerably in ecotone habitats, and driven by selective forces such as predation and host plant changes over time. The study here demonstrates the importance of field-based study of butterfly morphology for understanding trait and environment relationships—but this work is limited by a lack of laboratory evidence for plasticity for this particular case. Experimental approaches would well supplement these results and have the potential to address many of the open questions posed here. Such an integrated approach in the field and laboratory will help in understanding the persistence and the risk of extinction of species across diverse and species-rich ecosystems in Central Africa (Dongmo et al. 2017) and provide strategies that can be used by conservation biologists in the future under climate change (Bonebrake et al. 2018).

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