

## Chromosomal evolution in the South American Nymphalidae

KEITH S. BROWN, JR.<sup>1</sup>, ANDRÉ VICTOR LUCCI FREITAS<sup>1</sup>, NIKLAS WAHLBERG<sup>2</sup>,  
BARBARA VON SCHOULTZ<sup>3</sup>, ANJA O. SAURA<sup>3</sup> and ANSSI SAURA<sup>4</sup>

<sup>1</sup>*Museu de História Natural and Departamento de Zoologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil*

<sup>2</sup>*Department of Biology, Laboratory of Genetics, University of Turku, Finland and Department of Zoology, Stockholm University, Sweden*

<sup>3</sup>*Department of Biological and Environmental Sciences, University of Helsinki, Finland*

<sup>4</sup>*Department of Molecular Biology, Umeå University, Umeå, Sweden*

**Keith S. Brown, Jr., André Victor Lucci Freitas, Niklas Wahlberg, Barbara von Schoultz, Anja O. Saura and Anssi Saura 2007.** Chromosomal evolution in the South American Nymphalidae. — *Hereditas* 144: 137–148. Lund, Sweden. eISSN 1601-5223. Received June 11, 2007. Accepted June 11, 2007

We give the chromosome numbers of about 80 species or subspecies of Biblidinae as well as of numbers of neotropical Libytheinae (one species), Cyrestinae (4) Apaturinae (7), Nymphalinae (about 40), Limenitidinae (16) and Heliconiinae (11). *Libytheana* has about  $n=32$ , the Biblidinae, Apaturinae and Nymphalinae have in general  $n=31$ , the Limenitidinae have  $n=30$ , the few Argynnini  $n=31$  and the few species of Acraeni studied have also mostly  $n=31$ . The results agree with earlier data from the Afrotropical species of these taxa. We supplement these data with our earlier observations on Heliconiini, Danainae and the Neotropical Satyroid taxa. The lepidopteran modal  $n=29–31$  represents clearly the ancestral condition among the Nymphalidae, from which taxa with various chromosome numbers have differentiated. The overall results show that Neotropical taxa have a tendency to evolve karyotype instability, which is in stark contrast to the otherwise stable chromosome numbers that characterize both Lepidoptera and Trichoptera.

Anssi Saura, Department of Molecular Biology, Umeå University, SE-901 87 Umeå, Sweden. E-mail: [anssi.saura@molbiol.umu.se](mailto:anssi.saura@molbiol.umu.se)

The lepidopterans are characterized by stable chromosome numbers. The majority of butterflies and moths have a haploid chromosome number ranging from  $n=29$  to 31, with  $n=31$  found as the most common number across Lepidoptera from the Micropterygoidea all the way to butterflies (ROBINSON 1971; WHITE 1973; WERNER 1975). The lepidopteran chromosomes are small and have a nearly holokinetic structure. This should make fragmentation and translocations easy, since spindle fibers can always attach to a kinetochore (BAUER 1967). Nevertheless, stability prevails. Lycaenidae is the only family known to have a modal number ( $n=24$ ) of its own. Some lycaenids and representatives of the Palearctic satyrine genus *Erebia* have a series of allopatric species differing in karyotype (WHITE 1978; LORKOVIĆ 1990). Different chromosome numbers have been shown to reinforce a process of speciation that may originally have evolved through isolating mechanisms other than chromosome number (WIEMERS 2003; LUKHTANOV et al. 2005).

Nymphalidae is the largest family of butterflies with some 6500 species worldwide (VANE-WRIGHT 2003). Their diversity is highest in the Neotropics with some 3000 species, but they range to the Arctic and to all parts of world habitable to butterflies. In the surge of interest to elucidate the phylogeny and evolution of

nymphalids, morphological and molecular approaches have been used in combination (FREITAS and BROWN 2004; WAHLBERG et al. 2005b; JIGGINS et al. 2006; PEÑA et al. 2006; SIMONSEN et al. 2006; WAHLBERG 2006). This paper adds a chromosomal dimension: it is the final part of a series on the chromosomal evolution in neotropical Nymphalidae.

The earlier papers in this series have covered the chromosomes of the tribe Heliconiini (SUOMALAINEN and BROWN 1984; BROWN et al. 1992), the subfamilies Danainae and Ithomiinae (BROWN et al. 2004) and Charaxinae, Morphinae and Satyrinae (BROWN et al. 2007). We cover here observations on the chromosome numbers of neotropical representatives of the subfamilies Libytheinae, Biblidinae, Apaturinae, Nymphalinae, Limenitidinae and the tribes Argynnini and Acraeni of the Heliconiinae. FREITAS and BROWN (2004) and WAHLBERG et al. (2003, 2005b) have revised the overall taxonomy of the above groups.

### *The butterflies*

Libytheinae is a small subfamily, represented by the genus *Libytheana* in the New World, while the subfamily Biblidinae comprises more than 300 species placed in 30 genera. The monophyly of Biblidinae is well supported (FREITAS and BROWN 2004;

WAHLBERG et al. 2005a, 2005b), but the internal relationships within this subfamily are not completely resolved yet. Most of the tribes of Biblidinae are exclusively neotropical, while the Biblidini and Epicalini have paleotropical representatives as well. The larvae of most tribes feed on Euphorbiaceae (in particular on *Dalechampia* vines) but larvae of the Epiphilini and Callicorini feed on Sapindaceae (ARMBRUSTER 1997) and the genus *Eunica* (Epiphilini) is known to feed on a variety of different plant families (DEVRIES 1987; FREITAS and OLIVEIRA 1992; FREITAS et al. 1997). The larvae of many species of Biblidinae are known to construct frass chains that protect them against “walking” predators (DEVRIES 1987; FREITAS and OLIVEIRA 1996). The adults are medium to large butterflies that tend to be concentrated in small areas around the larval host plants (DEVRIES and WALLA 2001). The males perch; they may either have a cryptic color pattern that mimics tree bark (MONGE-NAJERA et al. 1998) or a conspicuous color pattern that suggests both aposematic coloration and/or mimicry. They are attracted to rotting fruit, fermented sap and dung (DEVRIES 1987).

DARWIN (1839) described the cracking sound of *Hamadryas* (*Papilio*) *feronia*. In contrast to the stridulatory sounds of most insects, the sound of *Hamadryas* is percussive. The butterflies use it as a means of communicating with conspecifics. Only the males have a forewing hearing organ, which is well developed in the Biblidinae and Satyrinae (YACK et al. 2000).

While the Apaturinae are a rather little known, mainly Asian group, represented in the Neotropics by two genera, the Nymphalinae are a diverse group indeed. The adults feed on nectar, some on rotting fruits and dung; many males “puddle” on moist soil. The larvae live on a variety of host plants, and adults of several species (e.g. *Siproeta stelenes* and *Eresia* spp.) are Batesian mimics of Ithomiini, Acraeini and Heliconiini.

Among the Nymphalinae, the Neotropical species of Melitaeini are related to Holarctic *Euphydryas* and *Melitaea*, the population structure of which has been extensively studied elsewhere (EHRlich and HANSKI 2004). The larvae of neotropical species feed mainly on Asteraceae and Acanthaceae (FREITAS 1991; WAHLBERG 2001); the adults feed on nectar and moist soil.

The Limenitidinae are represented in South America only by the large and diverse genus *Adelpha* (WILLMOTT 2003). The Heliconiinae are represented by three tribes in South America. BROWN et al. (1992) have published the chromosome numbers of Heliconiini. The Acraeini are entirely tropical, but even if

they are quite diverse in South America, their diversity is greatest in Africa. The distasteful adults are extensively involved in Müllerian and Batesian mimicry complexes. Larvae feed on Asteraceae in the New World, and all life stages are cyanogenic. In a like fashion, the main distribution of the tribe Argynnini is outside of South America, with two genera, *Euptoieta* and *Yramea*, present in South America.

There is growing evidence that nymphalids originated in the Neotropics (PEÑA et al. 2006; WAHLBERG 2006), although more detailed analyses of the entire family are needed. The hypothesis that new modal numbers (BROWN et al. 1992, 2004, 2007) seen in Heliconiini, Ithomiini and the satyroid groups are derived from the lepidopteran modal numbers  $n=29-31$  has been based on the assumption that the Libytheinae, the basal group of nymphalids (FREITAS and BROWN 2004; PEÑA et al. 2006), basal Heliconiinae (BROWN et al. 1992), and several other groups like Biblidinae, Apaturinae, Nymphalinae and Limenitidinae will have the lepidopteran modal  $n=31$  as the most common chromosome number. We here test this hypothesis and report on the chromosome numbers of these groups, with a strong emphasis on Biblidinae. The overall aim is to throw light on the evolution of the Nymphalidae.

## MATERIAL AND METHODS

Keith Brown has collected the butterflies in different parts of South America mainly during the 1970s and 1980s. The collection localities are given in the table in the Results section; often several collection sites are grouped together to give an overall area.

The gonads of butterflies were prepared as described in detail by BROWN et al. (1992) and stored for variable lengths of time until subjected to sectioning, staining and microscopy. Barbara von Schoultz did the practical laboratory work in the 1980s up to the year 1994. Dr. Esko Suomalainen of the Department of Genetics of the University of Helsinki, Finland, checked the chromosome number counts. A protracted illness and finally the death of Dr. Esko Suomalainen caused a break in the project so that the material was left unpublished. Anja O. Saura and Anssi Saura have, together with Keith Brown, Niklas Wahlberg and André Freitas, put the material together.

The exact collecting localities, dates, voucher specimens and references to microscopy are stored at the Museu de História Natural of the Universidade Estadual de Campinas, SP, Brazil, while the original laboratory notebooks and chromosome slides are at the Finnish Museum of Natural History,

University of Helsinki, Finland. We have also included earlier chromosome counts reported by MAEKI and REMINGTON (1960), DE LESSE (1967a, 1970a, 1970), DE LESSE and BROWN (1971), WESLEY and EMMEL (1975) and FRANCINI (1989). The nomenclature follows the checklist of LAMAS (2004), updated with phylogenetic results from FREITAS and BROWN (2004), WAHLBERG et al. (2003, 2005b) and PEÑA et al. (2006).

## RESULTS

Table 1 gives the chromosome numbers for two taxa of neotropical Libytheinae, 80 of Biblidinae, 7 of Apaturinae, 40 of Nymphalinae, 4 of Cyrestinae, 16 of Limenitidinae and 11 of Heliconiinae. The Libytheinae have either  $n=31$  or a slightly higher number. The few Cyrestinae studied have  $n=32$ . Within the Biblidinae, many samples of a single *Biblis* species all have  $n=28$ ; while the other species in Biblidini have  $n=31$  but there are exceptions with numbers one half of 30–31, i.e.  $n=14$ –15 even within a single species. The Ageroniini are characterized by  $n=31$  and so are the Epiphilini; again there are numbers half that ( $n=12$ –14) and one (*Temenis* sp.) with half of  $n=14$ , namely  $n=7$ . The Eubagini have numbers slightly lower than  $n=31$ , i.e.  $n=28$ –30, while the Callicorini have  $n=31$  or numbers close to this.

The Apaturinae and Nymphalinae have in general numbers about  $n=31$ . Two species of *Baeotus* are an exception: they have  $n=14$  and 15. Limenitidinae have  $n=30$  as the most common number, again with occasional  $n=15$  and once as low as  $n=11$ . Within the Heliconiinae, the few Argynnini all have  $n=31$ , and so have the five species of Acraeini studied by Francini (chromosomes are clearly visible only for a few hours just before pupation); the two other counts,  $n=14$  and  $n=ca\ 150$ , have been made on adult males.

## DISCUSSION

### *Patterns in chromosome numbers*

The main result is that all the Nymphalidae studied here have the lepidopteran modal of  $n=29$ –31 as the most common chromosome numbers. This strengthens the hypothesis put forward in the earlier papers of this series that the  $n=21$  of the genus *Heliconius* (BROWN et al. 1992),  $n=28$  of Morphini,  $n=29$  of Brassolini,  $n=29$  of other Satyrinae (BROWN et al. 2007) and the variable numbers with  $n=14$  as the modal one of the Ithomiini (BROWN et al. 2004), the quite divergent numbers of the tribes Anaeni and Preponini of Charaxinae and the multitude seen in

the satyrines (BROWN et al. 2007) are all derived from the lepidopteran modal of  $n=29$ –31.

WHITE (1978, p. 74) pointed out that the mechanisms for reducing the chromosome number below  $n=29$ –31 have been far more efficient than ones leading to increases above it. There will, however, be a problem with telomeres, which have to be silenced lest they interfere with the achiasmatic meiosis of lepidopteran females. DE LESSE (1967a), SUOMALAINEN and BROWN (1984), BROWN et al. (2007), and LORKOVIĆ (1990) have pointed out that there seems to be a process of concerted fusion that involves all chromosomes. It would explain why chromosome numbers that are about one half of the modal number are frequently seen among nymphalids. NORDENSKIÖLD (1951) has observed a similar concerted halving of chromosome numbers in the plant genus *Luzula* that has a diffuse kinetochore structure resembling that of lepidopterans. In fact, the Ithomiini have a strong modal at  $n=14$ –16 and very few forms with  $n=29$ –31 (BROWN et al. 2004). *Tellervo* and Danainae represent evidently the plesiomorphic condition. Accordingly, Ithomiini descend from forms that already have had their chromosome set halved. Our results show that  $n=14$ –15 has evidently arisen repeatedly from  $n=29$ –31 in different branches of nymphalid phylogeny, often without apparent intermediates. *Temenis* sp. shows, in addition, that  $n=14$  may experience one more round of concerted fusion with  $n=7$  as a result. The chromosome numbers of neotropical Nymphalidae show both stable numbers in certain taxa and apparently irregular numbers in others. Low, again stable or unstable, numbers may characterize entire subfamilies, while numbers higher than  $n=31$  are relatively infrequent.

Numbers between the modal numbers and even fractions or multiples of them are harder to explain. SEILER (1925) observed that fragmentation gives rise to different numbers. The nearly holocentric nature of lepidopteran chromosomes that makes fragmentation feasible was not known then and he was unable to give an adequate explanation to what he saw.

In the list given by ROBINSON (1971, p. 589) all groups of lepidopterans other than lycaenids have a modal number of  $n=29$ –31. We have here observed a set of exceptions. If we project the chromosome numbers of Neotropical Nymphalids onto the phylogeny of WAHLBERG et al. (2003) we observe the pattern seen in Fig. 1.

Libytheinae, the proposed sister group of all other Nymphalidae, have  $n=31$ –32 (this study); Danaini have  $n=30$  as the modal number (BROWN et al. 2004) and their sister group Ithomiini has numbers ranging from  $n=5$  to 120 with a peak at  $n=14$  (BROWN et al.

Table 1. Haploid chromosome numbers for South American Nymphalidae. The taxon name used in the original reference is in parentheses. A comma between chromosome numbers shows that the numbers come from different individuals. Localities are grouped by region; a number at the end of locality codes indicates the number of populations sampled within a region. A lower case letter in parentheses indicates previous work (*a* = DE LESSE 1967a, *b* = DE LESSE 1970a, *c* = DE LESSE 1970b, *d* = DE LESSE and BROWN 1971, *e* = FRANCINI 1989, *f* = MAEKI and REMINGTON 1960, *g* = WESLEY and EMMEL 1975, *h* = EMMEL and ELIAZAR, unpubl.). Locality codes: AC = Acre (extreme western Brazil), AM = Amazonas (northwestern Brazil), AN = Andes of north-central Colombia, AV = Amazonas (southern Venezuela), BA = Bahia (eastern Brazil), BO = Bolivar (southern Venezuela), CC = Chocó (western Colombia), DF = Brasília (central Brazil), DR = Dominican Republic, EE = eastern Ecuador, ES = Espírito Santo (eastern Brazil), MG = Minas Gerais (central Brazil), MT = Mato Grosso (central Brazil), OX = Oaxaca (southern Mexico), PA = Pará (northern Brazil), RJ = Rio de Janeiro (southeastern Brazil), RO = Rondônia (western Brazil), TV = Táchira (southwestern Venezuela), VC = Valle de Cauca (western Colombia), VV = Villavicencio, Meta (eastern Colombia).

Genus	Species, subspecies	n =	No. studied pop./ind.	Locality
Family NYMPHALIDAE				
Subfamily LIBYTHEINAE				
<i>Libytheana</i>	<i>carinenta</i>	31+2 small	1/1	Mexico(b)
<i>Libytheana</i>	<i>carinenta bachmanii</i> (L. b.)	31	1/7	Mexico(f)
Subfamily DANAINAE; BROWN et al. (2004)				
Tribe Danaini; BROWN et al. (2004)				
Tribe Ithomiini; BROWN et al. (2004)				
Subfamily CHARAXINAE; BROWN et al. (2007)				
Subfamily SATYRINAE; BROWN et al. (2007)				
Subfamily CYRESTINAE				
<i>Marpesia</i>	<i>berania</i>	32	1/1	Colombia(a)
<i>Marpesia</i>	<i>corinna</i>	33	1/2	Bolivia(a)
<i>Marpesia</i>	<i>petreus</i>	32	1/2	Argentina(a)
<i>Marpesia</i>	<i>zerynthia (coresia)</i>	32	1/2, 1/1	Bolivia(a), Ecuador(a)
Subfamily BIBLIDINAE				
Tribe Biblidini				
<i>Biblis</i>	<i>hyperia</i>	28	1/2, 1/5	Argentina(a), Mexico(f)
<i>Mestra</i>	<i>dorcas apicalis</i> (M. a.)	31	1/1	Bolivia(a)
<i>Mestra</i>	<i>dorcas hersilia</i>	31	1/1	Tobago(g)
	(M. hypermestra cana)			
<i>Mestra</i>	<i>dorcas semifulva</i> (M. s.)	33	1/1	Colombia(a)
<i>Vila</i>	sp.	15	1/1	RO
Tribe Epicaliini				
<i>Catonephele</i>	<i>antinoe</i>	14	1/2	Guyane(c)
<i>Catonephele</i>	<i>chromis</i>	15	1/1	TV
<i>Catonephele</i>	<i>numilia</i>	15	1/1	Mexico(b)
<i>Catonephele</i>	<i>nyctimus</i>	23	1/1	OX
<i>Catonephele</i>	<i>orites</i>	21	1/1	TV
<i>Catonephele</i>	<i>salambria</i>	15	1/1	TV
<i>Cybdelis</i>	<i>phaesyla (phaesila)</i>	23	1/2	Bolivia(a)
<i>Eunica</i>	<i>alcmena flora</i>	30	1/2	MT
<i>Eunica</i>	<i>bechina</i>	28	1/1	DF(d)
<i>Eunica (Libythina)</i>	<i>cwierii</i>	31	1/1	DF(d)
<i>Eunica (Evonyme)</i>	<i>eburnea</i>	30	1/2	Argentina(a)
<i>Eunica</i>	<i>ingens</i>	30	2/4	MT, RO(h)
<i>Eunica</i>	<i>macris</i>	16	1/1	RO(h)
<i>Eunica</i>	<i>malvina</i>	14, 31	1/1, 1/1	MT2
<i>Eunica</i>	<i>monima</i>	31	2/2	MT, RO
<i>Eunica</i>	<i>mygdonia</i>	31	1/3	MT
<i>Eunica</i>	nr <i>eurota</i>	30	1/1	TV
<i>Eunica</i>	nr <i>orphise</i>	29	1/1	ES
<i>Eunica (Evonyme)</i>	<i>tatila</i>	26	1/3	Argentina(a)
<i>Eunica</i>	sp.	15	1/1	RO(h)
<i>Eunica</i>	sp.	29–30	1/1	MT

Table 1 (Continued)

Genus	Species, subspecies	n =	No. studied pop./ind.	Locality
<i>Eunica</i>	sp.	31	1/1	AN
<i>Eunica</i>	sp.	31	1/1	RO(h)
<i>Myscelia</i>	<i>orsis</i>	24	1/1	RJ(d)
<i>Nessaea</i>	<i>batesii</i>	11	1/1	Guyane(c)
<i>Nessaea</i>	<i>hewitsonii</i>	7	1/1	La Macarena, Colombia(h)
<i>Nessaea</i>	<i>obrinus</i>	7	1/2	AM(h)
<i>Nessaea</i>	<i>obrinus</i>	8	1/1	BO
Tribe Ageroniini				
<i>Ectima</i>	<i>lirides</i>	16	1/1	AM
<i>Hamadryas</i>	<i>amphinome</i>	31	1/1	Colombia(a)
<i>Hamadryas</i>	<i>arinome</i>	31	1/1	Colombia(a)
<i>Hamadryas</i>	<i>epinome</i>	31	1/2	Argentina(a)
<i>Hamadryas</i>	<i>feronia</i>	31	1/1	Guyane(c)
<i>Hamadryas</i>	<i>glauconome</i>	31	1/2	Mexico(f)
<i>Hamadryas</i>	<i>guatemalena</i>	31	1/1	Mexico(b)
<i>Hamadryas</i>	<i>laodamia</i> ( <i>Peridroma arethusa</i> )	31	1/2	Guatemala(b)
<i>Hamadryas</i>	sp.	30	1/1	RO
<i>Panacea</i>	<i>procilla</i>	31	1/1	VC
<i>Panacea</i>	sp.	31	1/3	CC
Tribe Epiphilini				
<i>Asterope</i>	sp. nr <i>markii</i>	10	1/1	AC
<i>Asterope</i>	sp.	10	1/1	AV
<i>Epiphile</i>	<i>adrasta</i>	29	2/2	Guatemala(b), Mexico(b)
<i>Epiphile</i>	<i>hubneri</i> ( <i>huebneri</i> )	32	1/1	MG(d)
<i>Epiphile</i>	<i>orea</i>	32	1/1	DF
<i>Epiphile</i>	<i>orea</i>	32, 34	1/2	Argentina(a)
<i>Nica</i> ( <i>Pseudonica</i> )	<i>flavilla</i>	54	1/1	Guatemala(b)
<i>Peria</i>	<i>lamis</i>	23	1/1	EE
<i>Pyrrhogyra</i>	<i>neaerea ophni</i>	29	1/1	ES
<i>Pyrrhogyra</i>	<i>neaerea ophni</i>	31	1/1	BA
<i>Pyrrhogyra</i>	<i>otolais</i> ( <i>nasica</i> )	30	1/1	Colombia(a)
<i>Pyrrhogyra</i>	sp.	30	1/1	EE
<i>Temenis</i>	<i>laothoe</i>	11	1/1	MT(h)
<i>Temenis</i>	<i>laothoe</i>	12	2/3	DF, RO
<i>Temenis</i>	<i>laothoe</i>	13	3/5	Argentina(a), MG, PA
<i>Temenis</i>	<i>laothoe</i> (dark)	14	1/1	EE
<i>Temenis</i>	<i>laothoe</i>	14	1/1	VV
<i>Temenis</i>	<i>laothoe bahiana</i>	13 (2 small)	1/3	BA
<i>Temenis</i>	<i>pulchra</i>	27	2/3	VC, VV
<i>Temenis</i>	sp. nr <i>huebneri</i>	7	1/1	EE
Tribe Eubagini				
<i>Dynamine</i>	<i>agacles</i>	ca 29	1/1	Argentina(a)
<i>Dynamine</i>	<i>arene</i>	30	1/1	Tobago(g)
<i>Dynamine</i>	<i>athemon</i>	29	1/4	Argentina(a)
<i>Dynamine</i>	<i>coenus</i>	30	1/1	Argentina(a)
<i>Dynamine</i>	<i>myrrhina</i>	29	1/1	Argentina(a)
<i>Dynamine</i>	<i>postverta</i> ( <i>myllita</i> )	28	1/2	Guatemala(b)
<i>Dynamine</i>	<i>postverta</i> ( <i>myllita</i> )	29	2/4	Argentina(a), Guatemala(b)
<i>Dynamine</i>	<i>tithia</i>	30	1/1	Argentina(a)
<i>Dynamine</i>	<i>tithia salpensa</i> ( <i>D. s.</i> )	30	1/2	Bolivia(a)
Tribe Callicorini				
<i>Callicore</i>	<i>hydaspes</i>	30	1/2	Argentina(a)
<i>Callicore</i>	<i>hydaspes</i>	30–31	1/1	Argentina(a)
<i>Callicore</i>	<i>lyca</i>	31	1/1	Ecuador(a)
<i>Callicore</i>	<i>tolima</i>	21	1/1	Ecuador(a)
<i>Diaethria</i>	<i>anna</i> ( <i>annua</i> )	31	1/1	Mexico(b)
<i>Diaethria</i>	<i>candrena</i>	31	1/5	Argentina(a)
<i>Diaethria</i>	<i>clymena</i>	31	2/2	Argentina(a), Ecuador(a)

Table 1 (Continued)

Genus	Species, subspecies	n =	No. studied pop./ind.	Locality
<i>Diaethria</i>	<i>clymena marchalii</i> ( <i>D. m.</i> )	31	1/3	Colombia(a)
<i>Haematera</i> ( <i>Callidula</i> )	<i>pyrame</i>	30	1/2	Argentina(a)
<i>Haematera</i>	<i>pyrame</i>	30–31	1/1	RJ(h)
<i>Mesotaenia</i> ( <i>Perisama</i> )	<i>vaninka</i>	31	1/3	Bolivia(a)
<i>Orophila</i> ( <i>Perisama</i> )	<i>cardases</i>	31	1/1	Ecuador(a)
<i>Perisama</i>	<i>bomplandii</i>	31	1/3	Ecuador(a)
<i>Perisama</i>	<i>humboldtii</i>	31	1/1	Ecuador(a)
<i>Perisama</i>	<i>lebasii hilara</i> ( <i>P. h.</i> )	31–32	1/1	Bolivia(a)
<i>Perisama</i>	<i>morona</i>	31	1/2	Bolivia(a)
<i>Perisama</i>	<i>oppelii</i>	31	1/1	Ecuador(a)
Subfamily APATURINAE				
<i>Asterocampa</i>	<i>leilia</i>	31	1/1	Mexico(f)
<i>Doxocopa</i>	<i>cyane</i>	32–33	1/1	Ecuador(a)
<i>Doxocopa</i>	<i>elis</i>	31	1/1	Bolivia(a)
<i>Doxocopa</i>	<i>laurentia</i> ( <i>seraphina</i> )	31	1/1	Argentina(a)
<i>Doxocopa</i>	<i>linda</i>	32	1/1	Argentina(a)
<i>Doxocopa</i>	<i>pavon</i>	33–34	1/1	Mexico(b)
<i>Doxocopa</i>	sp. nr <i>agathina</i>	31	1/1	EE
Subfamily NYMPHALINAE				
Tribe Coeini				
<i>Baeotus</i>	<i>deucalion</i>	15	2/2	EE, RO(h)
<i>Baeotus</i>	sp.	14	1/1	CC
<i>Historis</i> ( <i>Coea</i> )	<i>acheronta</i>	31	1/1	Ecuador(a)
Tribe Nymphalini				
<i>Colobura</i>	<i>dirce</i>	31	1/2	RJ(d)
<i>Hypanartia</i>	<i>bella</i>	31	1/3	Argentina(a)
<i>Hypanartia</i>	<i>dione</i>	31	1/1	Bolivia(a)
<i>Hypanartia</i>	<i>kefersteini</i>	31	1/1	Bolivia(a)
<i>Hypanartia</i>	<i>lethe</i>	31	2/3	Argentina(a), Ecuador(a)
<i>Smyrna</i>	<i>blomfieldia</i>	31	1/2	MG
<i>Tigridia</i>	<i>acesta latifascia</i>	30	1/1	DF(d)
<i>Vanessa</i>	<i>carye</i>	31	1/1	Argentina(a)
<i>Vanessa</i>	<i>virginiensis</i>	31	1/1	Argentina(a)
Tribe Victorinini				
<i>Anartia</i>	<i>amathea</i>	30–31	1/1	Argentina(a)
<i>Anartia</i>	<i>amathea</i>	31	3/10	Argentina(a), Trinidad2(g)
<i>Anartia</i>	<i>amathea</i>	32	1/1	Argentina(a)
<i>Anartia</i>	<i>fatima</i>	31	1/2	Mexico(f)
<i>Anartia</i>	<i>jatrophae</i>	31	6/8	Bolivia(a), Colombia(a), Guyane(c), Mexico(f), Trinidad2(g)
<i>Metamorpha</i>	<i>elissa</i> ( <i>sulpicia</i> )	31	1/3	Ecuador(a)
<i>Siproeta</i>	<i>epaphus</i>	31	1/3	Ecuador(a)
<i>Siproeta</i> ( <i>Metamorpha</i> )	<i>stelenes</i>	31	3/4	Colombia(a), Ecuador(a), Mexico(f)
Tribe Junoniini				
<i>Junonia</i>	<i>coenia</i>	31	1/1	Mexico(f)
<i>Junonia</i>	<i>evarete</i>	31	2/4	Ecuador(a), RJ
<i>Junonia</i> ( <i>Precis</i> )	<i>evarete zonalis</i> ( <i>lavina</i> z.)	31	3/3	Mexico(f), Trinidad2(g)
<i>Junonia</i>	<i>vestina</i>	31	1/1	Argentina(a)
<i>Junonia</i>	<i>vestina livia</i>	31	1/1	Ecuador(a)
Tribe Melitaeini				
<i>Anthanassa</i> ( <i>Phyciodes</i> )	<i>frisias hermas</i>	31	1/1	Argentina(a)
<i>Castilia</i> ( <i>Phyciodes</i> )	<i>eranites</i>	31	1/1	Colombia(a)
<i>Chlosyne</i>	<i>gaudialis</i>	31	1/1	Mexico(b)
<i>Chlosyne</i>	<i>hippodrome</i>	31	1/1	Mexico(b)
<i>Chlosyne</i>	<i>janais</i>	31	2/4	Mexico2(b)
<i>Chlosyne</i>	<i>lacinia adjutrix</i>	31	1/1	Mexico(b)

Table 1 (Continued)

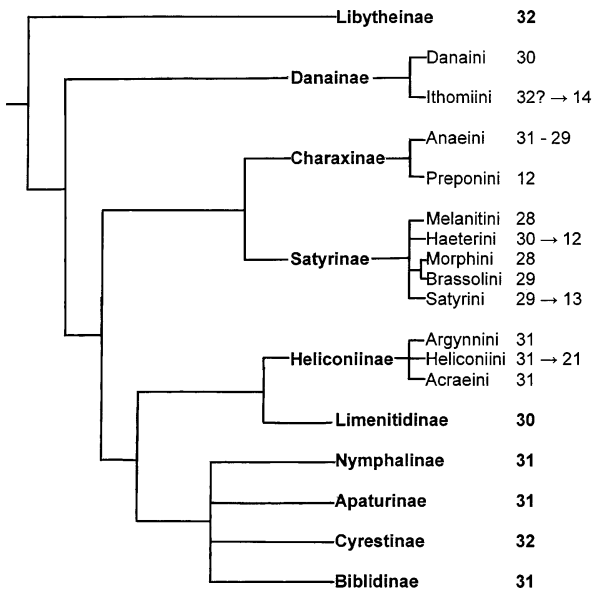
Genus	Species, subspecies	n =	No. studied pop./ind.	Locality
<i>Chlosyne</i>	<i>lacinia lacinia</i>	31	1/1	Mexico(b)
<i>Chlosyne</i>	<i>narva</i>	ca 31	1/1	Colombia(a)
<i>Eresia</i>	<i>datis moesta</i> ( <i>Phyciodes m.</i> )	32	1/3	Ecuador(a)
<i>Eresia</i>	<i>datis moesta</i> ( <i>Phyciodes m.</i> )	33, 33–34	1/2	Ecuador(a)
<i>Eresia</i>	<i>datis margaretha</i> ( <i>Phyciodes m.</i> )	ca 34–35	1/1	Colombia(a)
<i>Eresia</i> ( <i>Phyciodes</i> )	<i>emerantia</i>	31	1/1	Colombia(a)
<i>Eresia</i> ( <i>Phyciodes</i> )	<i>lansdorfi</i>	31	2/2	Argentina(a), MG(d)
<i>Ortilia</i> ( <i>Phyciodes</i> )	<i>ithra</i>	31	1/1	Argentina(a)
<i>Telenassa</i> ( <i>Phyciodes</i> )	<i>teletusa</i>	31	1/1	Argentina(a)
Subfamily LIMENITIDINAE				
<i>Adelpha</i>	<i>alala</i>	42, 45	1/2	Bolivia(a)
<i>Adelpha</i>	<i>cocala</i>	27	1/1	Ecuador(a)
<i>Adelpha</i>	<i>c. cocala</i> ( <i>c. urraca</i> )	15, 16	1/2	MT
<i>Adelpha</i>	<i>cocala didia</i> ( <i>c. riola</i> )	30	1/1	RJ(d)
<i>Adelpha</i>	<i>cytherea</i>	30	1/2	Colombia(a)
<i>Adelpha</i>	<i>epione</i>	33	1/1	VC
<i>Adelpha</i>	<i>epione</i> ssp.	32–33	1/1	EE
<i>Adelpha</i>	<i>justina valentina</i> ( <i>A. v.</i> )	30	1/1	Ecuador(a)
<i>Adelpha</i>	<i>lycorias lara</i> ( <i>A. lara</i> )	30	1/1	Ecuador(a)
<i>Adelpha</i>	<i>malea goyama</i> ( <i>A. g.</i> )	30	1/1	Argentina(a)
<i>Adelpha</i>	<i>mesentina</i>	30	1/2	AM
<i>Adelpha</i>	<i>mythra</i>	30	1/1	RJ(d)
<i>Adelpha</i>	<i>saundersii</i>	30	1/1	Bolivia(a)
<i>Adelpha</i>	<i>serpa</i>	11	1/2	ES
<i>Adelpha</i>	<i>syma</i>	29	1/1	MG(d)
<i>Adelpha</i>	<i>thessalia indefecta</i> ( <i>A. mincia</i> )	30	1/2	Argentina(a)
Subfamily HELICONIINAE				
Tribe Argynnini				
<i>Euptoieta</i>	<i>hegesia</i>	31	2/3	Bolivia(a), Mexico(f)
<i>Euptoieta</i>	<i>hortensia</i>	31	1/2	Argentina(a)
<i>Yramea</i>	<i>cytheris</i>	31	2/4	Argentina(a), Chile(a)
<i>Yramea</i>	<i>lathonioides</i>	31	1/1	Chile(a)
Tribe Acraeini				
<i>Abananote</i> ( <i>Actinote</i> )	<i>erinome</i>	ca 150	1/1	Bolivia(a)
<i>Actinote</i>	<i>carycina</i>	31	several	SP(e)
<i>Actinote</i>	<i>melanisans</i>	31	several	SP(e)
<i>Actinote</i>	<i>parapheles</i>	31	several	SP(e)
<i>Actinote</i>	<i>pellenea</i>	31	several	SP(e)
<i>Actinote</i>	<i>thalia pyrrha</i> ( <i>A. p.</i> )	31	several	SP(e)
<i>Altinote</i> ( <i>Actinote</i> )	<i>alcione corduba</i>	14	1/1	Bolivia(a)
Tribe Heliconiini; SUOMALAINEN and BROWN (1984), BROWN et al. (1992)		31 →21		

2004). The two tribes of Charaxinae have quite different distributions of numbers (BROWN et al. 2007). The Anaecini have a peak at  $n=31$ , followed by a descending series with many numbers in  $n=26$  through 30 and a minor peak at  $n=21$ , all the way to  $n=6$ , while the Preponini have a peak at  $n=12$  with a single number above  $n=19$ .

Among Satyrinae (BROWN et al. 2007), the Morphini have a peak at  $n=28$ , while the Brassolini have an equally distinctive peak at  $n=29$ . The other tribes of Satyrinae have a weak modal of  $n=29$  (BROWN

et al. 2007), starting with the basal groups with  $n=29$  relatively common, followed with the first clade of Pronophilina (PEÑA et al. 2006) which has  $n=29$  fixed, followed by the second clade that has an uneven distribution resembling the one of Euptychiina that has all numbers between  $n=6$  and  $n=31$  present at least once, with  $n=13$  as the most common one but without any clear modal number.

The next clade is made up of Heliconiinae (including Argynnini, Heliconiini and Acraeini) and Limenitidinae. The samples for Argynnini and Acraeini are



**Fig. 1.** The chromosome numbers of Neotropical Nymphalids projected onto the phylogeny of FREITAS and BROWN (2004) or WAHLBERG et al. (2003). The most common chromosome number within each taxon is given; the arrows indicate the direction of change from primitive to derived forms.

small but they seem to be almost fixed for  $n=31$ , while the Heliconiini show an evolution away from  $n=29-31$  to a new modal number (SUOMALAINEN and BROWN 1984; BROWN et al. 1992). The basal genus, *Philaethria*, is made up of species with  $n=12$  up to  $n=88$ . Two of the species have  $n=29$ , which is also found in the other primitive genus, *Podotricha*, which again has a species with  $n=9$ . The next clades are genera with  $n=31$ , followed through a series (*Neruda*, *Laparus*) that go down from  $n=32$  to  $n=19-21$ . The large genus *Heliconius* is, with the exception of the most derived, pupal mating clade, stabilized into  $n=21$ . With the exception of *H. hewitsoni* that has  $n=21$ , the pupal mating species represent an ascending series up to  $n=62$ .

The Limenitidinae have a strong modal  $n=30$  (this study) and the Nymphalinae an even stronger  $n=31$  and this is also the case in the small sample of Apaturinae that we have in this study. Finally the Biblidinae have a strong modal number of  $n=31$  (31 out of 80 taxa) followed with  $n=30$  and  $n=29$ . We may also note that numbers about half that are relatively common with a total of 13 counts between  $n=13$  and  $n=16$ .

WAHLBERG (2006) has estimated that the basal groups of Nymphalinae diverged at about the K/T boundary, i.e. about 65 million years ago and the age of Nymphalidae is older than 70 million years. This

gives us a handle to assess whether the modal number represents a primitive condition rather than an equilibrium karyotype in the sense of WHITE (1973) to which the chromosome number will return after having been perturbed. These two concepts need, of course, not be mutually exclusive. Many of the basal subfamilies and tribes of nymphalids have the modal  $n=29-31$ . Again, BROWN et al. (2004) argued that the Ithomiini evidently descend from an ancestor that has already had the chromosome number halved to about  $n=14-15$ . The minor peak seen at about  $n=7-8$  seen among them results from further concerted fusion of all chromosomes. Given that the nymphalid subfamilies and tribes have diverged from each other tens of millions of years ago (WAHLBERG 2006), it is unlikely that there is selection that will restore  $n=31$  once it has been perturbed. Evidently  $n=29-31$  represents the ancestral condition of Nymphalidae.

### Chromosomes in speciation

The pairing and segregation of chromosomes at meiosis is a component of fertility selection, a constituent of postzygotic isolation and speciation (DOBZHANSKY 1968). Chromosome number changes have been shown to give rise to reinforcement in satyrine speciation (LORKOVIĆ 1958). The factors underlying reinforcement are being studied with molecular methods: LUKHTANOV and DANTCHENKO (2002) and LUKHTANOV et al. (2005) have studied the behavior at meiosis of the chromosomes of Lycaenidae, in particular species with extremely high chromosome numbers, again, WOLF et al. (1997) have observed meiosis in lepidopterans with low chromosome numbers. We have here an acraeina that may have an extremely high chromosome number ( $n=150$ ) and we have reported both very low and as high or higher numbers also in other Nymphalids (BROWN et al. 1992, 2004, 2007).

DE LESSE (1966, 1967b, 1968) and DE LESSE and Condamin (1962, 1965) have published chromosome numbers of African representatives of the neotropical Nymphalid groups that we report here. In general the African Nymphalinae have  $n=31$ , the Limenitidinae have a peak at  $n=30$ , like in South America; the few Biblidinae at  $n=31$  and the single *Libythea* species  $n=31$ . The sample of 18 species of African Acraeinae has a peak at  $n=31$  but nine species have numbers higher than that and one species has  $n=137$ , comparable to *Abananote erinome* of Bolivia (this paper). FRANCINI (1989) has, in our opinion convincingly, demonstrated that in the study of chromosome numbers of Acraeini one should only look at early prepupae; all other stages of development yield



nonreproducible results. Nevertheless, given the expertise and perspicuity of de Lesse, we think that his two similar sets of observations carry weight and should not be disregarded without rechecking.

ROBINSON (1971) has made an extensive compilation of worldwide chromosome numbers of lepidopterans. Virtually every group of Nymphalids from North America, Europe, Asia and Australia has a modal number at  $n = 30-31$ . The only exceptions are the African Charaxines that have a peak at  $n = 25-26$  and the African Satyrines that have a modal of  $n = 28$  (BROWN et al. 2007). With these two exceptions, all other Nymphalid groups with modal chromosome numbers different from the general lepidopteran modal of  $n = 29-31$  are Neotropical. LORKOVIĆ (1990) pointed out, on the basis of a limited material, that the tropical Satyrinae tend to have lower chromosome numbers than the  $n = 29$  that characterizes them in the rest of the world. Given that there is no crossing over in the females, chromosome numbers may represent a way to adapt to tropical conditions through adjusting recombination. We doubt that the hypothesis of LORKOVIĆ (1990) needs to be discussed further; e.g. most tropical and temperate *Drosophila* species lack recombination in the heterogametic sex but all have low chromosome numbers.

#### Modes of selection

DOBZHANSKY (1950) argued that in the physically mild environments of the tropics the interrelationships between competing and symbiotic species or biotic interactions in general are the agents of natural selection, while in the harsher environments of the temperate zone and beyond physical factors drive evolution. JANZ et al. (2006) have shown that host plant diversification drives evolution in Nymphalidae. Mimicry is another obvious case point. Among the groups discussed here, Danainae, several Charaxinae and Satyrinae, Heliconiinae, many Nymphalinae and some Biblidinae are involved in mimicry rings either as movers or followers. Ithomiini, Charaxinae, Satyrinae and some Heliconiini are characterized through chromosomal instability. The large genus *Heliconius* stands out among these mimetic forms, as it is almost fixed for the new modal  $n = 21$ . GILBERT (2003) shows that there is extensive between species mating that explains the striking convergence of Müllerian mimetic patterns across the genus.

Hybridization is a potential mechanism that could give rise to chromosomal changes within and among closely related species (MALLET 2007). Our material (BROWN et al. 1992) examined includes certain *Heliconius* hybrids found in nature. Some of them

are hybrids between different morphs of one heliconiine species (*Eueides tales tales*  $\times$  *E. tales pythagoras*, *Heliconius clysonymus clysonymus*  $\times$  *H. clysonymus hygiana*, *H. sapho sapho*  $\times$  *H. sapho chocoensis*). One of them (*H. cydno*  $\times$  *H. melpomene*) is a species hybrid. In all these cases the parentals of the hybrid have the same chromosome number (even though the chromosome number of *E. tales pythagoras* is unknown). The chromosomes seem to pair in general in the normal fashion in the hybrid meiosis, indicating that the hybrids may well be fertile. In only one of the hybrids studied by us (*E. tales tales*  $\times$  *E. tales pythagoras*) all chromosomes do not pair in a part of the cells. The subspecies (morphs) and closely related species of *Heliconius* in general have the same chromosome number. Consequently their hybrids lack the obstacle for fertility conferred by different chromosome numbers of parent species. There is, indeed, good evidence for homoploid speciation in *Heliconius*: *H. heurippa* has originated as a hybrid between *H. melpomene* and *H. cydno* (MAVÁREZ et al. 2006). Here the hybrid phenotype isolates the hybrids from the parent species. Consequently, between species hybridization would be a force that stabilizes the chromosome numbers. As mentioned in the introduction, chromosome number change is in general expected to give rise to reproductive isolation and reinforcement (LUKHTANOV et al. 2005; KANDUL et al. 2007).

Another case in point is sexual selection. The males of the pupal mating clade of *Heliconius* mate with the female before she has eclosed from the pupa and consequently sexual selection is relaxed. GILBERT (2003) has suggested that sexual selection is a conservative force in the evolution of *Heliconius*. Our chromosomal results show that once sexual selection is removed, chromosome numbers become unstable, which is certainly compatible with GILBERT'S (2003) suggestion.

WAHLBERG et al. (2005b) and WAHLBERG (2006) have reconstructed the historical biogeography of the Nymphalinae. They conclude that the major clades have three centres of diversification, from which they have spread to the areas they now occupy. The pattern of chromosomal evolution that we have observed agrees with their conclusions: there have been several dispersal events from South America to other continents; again South America has received many taxa in particular from the Afrotropical and Nearctic regions. Interestingly, groups that may have invaded to the Neotropics from elsewhere (e.g. Argynnini and Melitaeini) seem to have retained the lepidopteran modal  $n = 31$ , while at least some clades of Satyrinae,

a putative Neotropical subfamily, have retained chromosomal instability and make use of it in speciation (LORKOVIĆ 1958).

### Conclusions

In conclusion, we have found that many Neotropical groups of Nymphalidae show extensive variation in their chromosome numbers, while chromosome numbers appear to be much more stable in the Holarctic region. Karyotypic instability characterizes entire subfamilies like Charaxinae or tribes or subtribes, like Ithomiini among danainae and Euptychiina among satyrines. We suggest that this instability is associated with speciation and it is driven through biotic interactions such as mimicry. Nymphalids are a well-studied group that could be an ideal material to solve the role of chromosomal change vs. stability in evolution. We call for studies on historical biogeography (WAHLBERG 2006) and molecular studies tied to cytology (LUKHTANOV et al. 2005; MAVÁREZ et al. 2006; GOMPERT et al. 2006; KANDUL et al. 2007) to re-establish through modern means the once flourishing but long neglected field of chromosome evolution.

*Acknowledgements* – K. S. Brown is grateful to Drs. Thomas Emmel and Peter Eliazar of the Dept of Zoology, Univ. of Florida at Gainesville, FL for comments and unpublished information, and to BIOTA-FAPESP (98/05101-8) for laboratory supplies and the Brazilian CNPq for research fellowships and for help in travel. André V. L. Freitas acknowledges FAPESP grants 00/01484-1 and 04/05269-9 and the BIOTA-FAPESP program (98/05101-8), the Brazilian CNPq (fellowship 300315/2005-8) and the National Science Foundation grant DEB-0527441. Niklas Wahlberg acknowledges support from the Swedish Research Council. The work of Barbara von Schoultz was supported by grants from the Natural Science Research Council of Finland to the late Dr. Esko Suomalainen. A. O. Saura is grateful to the Oskar Öflunds Stiftelse and A. Saura to the Royal Physiographic Society of Lund, Sweden, for support in travel.

### REFERENCES

- Armbruster, W. S. 1997. Exaptations link evolution of plant-herbivore and plant-pollinator interactions: a phylogenetic inquiry. – *Ecology* 78: 1661–1672.
- Bauer, H. 1967. Die kinetische Organisation der Lepidopteren-Chromosomen. – *Chromosoma* 22: 101–125.
- Brown, K. S. Jr., Emmel, T. C., Eliazar, P. J. et al. 1992. Evolutionary patterns in chromosome numbers in neotropical Lepidoptera I. Chromosomes of the Heliconiini (Family Nymphalidae: Subfamily Nymphalinae). – *Hereditas* 117: 109–125.
- Brown, K. S. Jr., von Schoultz, B. and Suomalainen, E. 2004. Chromosome evolution in neotropical Danainae and Ithomiinae (Lepidoptera). – *Hereditas* 141: 216–236.
- Brown, K. S. Jr., Freitas, A. V. L., von Schoultz, B. et al. 2007. Chromosomal evolution of South American frugivorous butterflies in the Satyroid clade Nymphalidae: Charaxinae, Morphinae and Satyrinae. – *Biol. J. Linn. Soc.* 88: 00–00.
- Darwin, C. 1839. *Voyages of the adventure and the Beagle*. Vol III Darwin. Journal and remarks 1983–1986 – Colburn, London.
- DeVries, P. J. 1987. *The butterflies of Costa Rica and their natural history*. Vol. I: Papilionidae, Pieridae, Nymphalidae. – Princeton Univ. Press.
- DeVries, P. J. and Walla, T. R. 2001. Species diversity and community structure in neotropical fruit-feeding butterflies. – *Biol. J. Linn. Soc.* 74: 1–15.
- Dobzhansky, Th. 1950. Evolution in the tropics. – *Am. Sci.* 38: 209–221.
- Dobzhansky, Th. 1968. On some fundamental concepts of Darwinian biology. – In: Dobzhansky, Th., Hecht, M. K. and Steere, W. C. (eds), *Evolutionary biology 2*. Appleton-Century-Crofts, New York, p. 1–34.
- Ehrlich, P. and Hanski, I. (eds) 2004. *On the wings of checkerspots /a model system for population biology*. – Oxford Univ. Press.
- Francini, R. B. 1989. *Biologia e ecologia das borboletas Actinote* (Lepidoptera, Nymphalidae, Acraeinae) na transição subtropical no sudeste do Brasil. – Dissertação de Mestrado em ecologia. Univ. Estadual de Campinas, Campinas, Brazil.
- Freitas, A. V. L. 1991. Variação morfológica, ciclo de vida e sistemática de *Tegosa claudina* (Eschscholtz) (Lepidoptera: Nymphalidae, Melitaeinae) no Estado de São Paulo. – *Rev. Bras. Entomol.* 35: 301–306.
- Freitas, A. V. L. and Oliveira, P. S. 1992. Biology and behavior of the neotropical butterfly *Eunica bechina* (Nymphalidae) with special reference to larval defence against ant predation. – *J. Res. Lepidopt.* 31: 1–11.
- Freitas, A. V. L. and Oliveira, P. S. 1996. Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. – *J. Anim. Ecol.* 65: 205–210.
- Freitas, A. V. L. and Brown, K. S. Jr. 2004. Phylogeny of the Nymphalidae (Lepidoptera). – *Syst. Biol.* 53: 363–383.
- Freitas, A. V. L., Brown, K. S. and Otero, L. D. 1997. Juvenile stages of *Cybdelis*, a key genus uniting the diverse branches of Eurytelinae (Lepidoptera, Nymphalidae). – *Trop. Lepidopt.* 8: 29–34.
- Gilbert, L. 2003. Adaptive identity through introgression in *Heliconius* wing patterns: evidence for a shared genetic “toolbox”. From synthetic hybrid zones and a theory of diversification. – In: Boggs, C. L., Watt, W. B. and Ehrlich, P. R. (eds), *Butterflies/ecology and evolution taking flight*. Univ. of Chicago Press, p. 281–318.
- Gompert, M. L. Z., Fordyce, J. A., Forister, M. L. et al. 2006. Homoploid hybrid speciation in an extreme habitat. – *Science* 314: 1923–1924.
- Janz, N., Nylin, S. and Wahlberg, N. 2006. Diversity begets diversity: host expansions and the diversification of plant-feeding insects. – *BMC Evol. Biol.* 6:4, doi: 10.1186/1471-2148-6-4.
- Jiggins, C. D., Mallarino, R., Willmott, K. R. et al. 2006. The phylogenetic pattern of speciation and wing pattern

- change in Neotropical *Ithomia* butterflies (Lepidoptera: Nymphalidae). – *Evolution* 60: 1454–1466.
- Kandul, N. P., Lukhtanov, V. A. and Pierce, N. E. 2007. Karyotypic diversity and speciation in *Agrodiaetus* butterflies. – *Evolution* 61: 546–559.
- Lamas, G. 2004. Libytheinae, Biblidinae through Heliconiinae. – In: Lamas G. (ed.), Atlas of Neotropical Lepidoptera, Checklist: Part 4A, Hesperioidea and Papilionoidea. – Scientific Publishers, Gainesville, p. 171, 234–274.
- de Lesse, H. 1966. Formules chromosomiques de quelques Lépidoptères Rhopalocères d'Afrique centrale. – *Ann. Soc. Entomol. France (N.S.)* 2: 349–353.
- de Lesse, H. 1967a. Les nombres de chromosomes chez les Lépidoptères Rhopalocères néotropicaux. – *Ann. Soc. Entomol. France (N.S.)* 3: 67–136.
- de Lesse, H. 1967b. Formules chromosomiques de Lépidoptères Rhopalocères d'Afrique centrale (supplément et rectificatif). – *Bull. Soc. Entomol. France* 72: 287–288.
- de Lesse, H. 1968. Formules chromosomiques de Lépidoptères Rhopalocères d'Uganda et du Kenya. – *Ann. Soc. Entomol. France (N.S.)* 4: 581–599.
- de Lesse, H. 1970a. Les nombres de chromosomes chez les Lépidoptères Rhopalocères en Amérique centrale et Colombie. – *Ann. Soc. Entomol. France (N.S.)* 6: 347–358.
- de Lesse, H. 1970b. Formules chromosomiques de quelques Lépidoptères Rhopalocères de Guyane. – *Ann. Soc. Entomol. France (N.S.)* 6: 849–855.
- de Lesse, H. and Brown, K. S. Jr. 1971. Formules chromosomiques de Lépidoptères Rhopalocères du Brésil. – *Bull. Soc. Entomol. France* 76: 131–137.
- de Lesse, H. and Condamin, M. 1962. Formules chromosomiques de quelques Lépidoptères Rhopalocères du Sénégal. – *Bull. l'IFAN* 24 Sér. A: 464–473.
- de Lesse, H. and Condamin, M. 1965. Formules chromosomiques de quelques Lépidoptères Rhopalocères du Sénégal et de Côte d'Ivoire. – *Bull. l'IFAN* 27 Sér. A: 1089–1094.
- Lorković, Z. 1958. Some peculiarities of spatially and sexually restricted gene exchange in the *Erebia tyndarus* group. – *Cold Spring Harbor Symp. Quant. Biol.* 23: 319–325.
- Lorković, Z. 1990. The butterfly chromosomes and their application in systematics. – In: Kudrna, O. (ed.), Butterflies of Europe. Vol. 2. Aula Verlag, Wiesbaden, p. 332–396.
- Lukhtanov, V. A. and Dantchenko, A. V. 2002. Principles of highly ordered arrangement of metaphase I bivalents in spermatocytes of *Agrodiaetus* (Insecta, Lepidoptera). – *Chromosome Res.* 10: 5–20.
- Lukhtanov, V. A., Kandul, N. P., Plotkin, J. B. et al. 2005. Reinforcement of pre-zygotic isolation and karyotype evolution in *Agrodiaetus* butterflies. – *Nature* 436: 385–389.
- Mallet, J. 2007. Hybrid speciation. – *Nature* 446: 279–283.
- Maeki, K. and Remington, C. L. 1960. Studies of the chromosomes of North American Rhopalocera. 4. Nymphalinae, Charaxidinae, Libytheinae. – *J. Lepidopt. Soc.* 14: 179–201.
- Mavárez, J. C., Salazar, A. and Bermingham, E. et al. 2006. Speciation by hybridization in *Heliconius* butterflies. – *Nature* 441: 868–871.
- Monge-Najera, J., Hernández, F., González, F. et al. 1998. Spatial distribution, territoriality and sound production by tropical cryptic butterflies (*Hamadryas*, Lepidoptera: Nymphalidae): implications for the industrial melanism debate. – *Rev. Biol. Trop.* 46: 297–330.
- Nordenskiöld, H. 1951. Cytotaxonomical studies in the genus *Luzula*. I. – *Hereditas* 37: 324–355.
- Peña, C., Wahlberg, N., Weingartner, E. et al. 2006. Higher level phylogeny of Satyrinae butterflies (Lepidoptera: Nymphalidae) based on DNA sequence data. – *Mol. Phylogenet. Evol.* 40: 29–49.
- Robinson, R. 1971. Lepidoptera genetics. – Pergamon Press.
- Seiler, J. 1925. Ergebnisse aus Kreuzungen von Schmetterlingsrassen verschiedener Chromosomenzahl. – *Arch. Julius-Klaus-Stiftung Vererb. Rassenhygiene* 1: 63–117.
- Simonsen, T. J., Wahlberg, N., Brower, A. V. Z. et al. 2006. Phylogeny of Argynnini: morphology, molecules and fritillaries: approaching a stable phylogeny for Argynnini (Lepidoptera: Nymphalidae). – *Insect Syst. Evol.* 37: 405–418.
- Suomalainen, E. and Brown, K. S. Jr. 1984. Chromosome numbers within *Philaethria* butterflies (Lepidoptera: Nymphalidae, Heliconiini). – *Chromosoma* 90: 170–176.
- Vane-Wright, R. I. 2003. Evidence and identity in butterfly systematics. – In: Boggs, C. L., Watt, W. B. and Ehrlich, P. R. (eds), Butterflies/ecology and evolution taking flight. Univ. of Chicago Press, p. 477–513.
- Wahlberg, N. 2001. The phylogenetics and biochemistry of host plant specialization in melitaeine butterflies (Lepidoptera: Nymphalidae). – *Evolution* 55: 522–537.
- Wahlberg, N. 2006. That awkward age for butterflies: insights from the age of the butterfly subfamily Nymphalidae (Lepidoptera: Nymphalidae). – *Syst. Biol.* 55: 703–714.
- Wahlberg, N., Weingartner, E. and Nylin, S. 2003. Towards a better understanding of the higher systematics of Nymphalidae (Lepidoptera: Papilionoidea). – *Mol. Phylogenet. Evol.* 28: 473–484.
- Wahlberg, N., Braby, M. F., Brower, A. V. Z. et al. 2005a. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. – *Proc. R. Soc. Lond. B* 272: 1577–1586.
- Wahlberg, N., Brower, A. V. Z. and Nylin, S. 2005b. Phylogenetic relationships and historical biogeography of tribes and genera in the subfamily Nymphalinae (Lepidoptera: Nymphalidae). – *Biol. J. Linn. Soc.* 86: 227–251.
- Werner, L. 1975. Zur Evolution des Karyotyps bei den Lepidopteren. Die Chromosomenzahlen der Noctuiden. – *Genetica* 45: 377–395.
- Wesley, D. J. and Emmel, T. C. 1975. The chromosomes of neotropical butterflies from Trinidad and Tobago. – *Biotropica* 7: 24–31.
- Wiemers, M. 2003. Chromosome differentiation and the radiation of the butterfly subgenus *Agrodiaetus* (Lepidoptera: Lycaenidae: Polyommatus)—a molecular phylogenetic approach. – PhD thesis, Univ. of Bonn.
- White, M. J. D. 1973. Animal cytology and evolution, 3rd ed. – Cambridge Univ. Press.
- White, M. J. D. 1978. Modes of speciation. – Freeman.
- Willmott, K. R. 2003. Cladistic analysis of the Neotropical butterfly genus *Adelpha* (Lepidoptera: Nymphalidae),

- with comments on the subtribal classification of Lime-  
nitidini. – *Syst. Entomol.* 28: 279–322.
- Wolf, K. W., Novák, K. and Marec, F. 1997. Kinetic  
organization of metaphase I bivalents in spermatogenesis  
of Lepidoptera and Trichoptera species with small  
chromosome numbers. – *Heredity* 79: 135–143.
- Yack, J. E., Otero, L. D., Dawson, J. W. et al. 2000. Sound  
production and hearing in the blue cracker butterfly  
*Hamadryas feronia* (Lepidoptera, Nymphalidae) from  
Venezuela. – *J. Exp. Biol.* 203: 3689–3702.