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Chromosome evolution in Neotropical butterflies

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We list the chromosome numbers for 65 species of Neotropical Hesperiidae and 104 species or subspecies of Pieridae. In Hesperiidae the tribe Pyrrhopygini have a modal n = 28, Eudaminae and Pyrgini a modal n = 31, while Hesperiinae have n = around 29. Among Pieridae, Coliadinae have a strong modal n = 31 and among Pierinae Anthocharidini are almost fixed for n = 15 while Pierini vary with n = 26 as the most common chromosome number. Dismorphiinae show wide variation. We discuss these results in the context of chromosome numbers of over 1400 Neotropical butterfly species and subspecies derived from about 3000 populations published here and in earlier papers of a series. The overall results show that many Neotropical groups are characterized by karyotype instability with several derived modal numbers or none at all, while almost all taxa of Lepidoptera studied from the other parts of the world have one of n = 29–31 as modal numbers. Possibly chromosome number changes become fixed in the course of speciation driven by biotic interactions. Population subdivision and structuring facilitate karyotype change. Factors that stabilize chromosome numbers include hybridization among species sharing the same number, migration, sexual selection and possibly the distribution of chromosomes within the nucleus.

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The butterflies have been the textbook example of stable chromosome numbers in animals. WHITE (1973, 1978) gives a histogram showing the chromosome numbers of about 740 species of butterflies. There is a distinct concentration of numbers around n = 29, 30 and 31, with well over half the species having one of these numbers and over a quarter, n = 31. The distribution is strongly skewed, with few numbers above n = 31 and many numbers below n = 29. WHITE (1978) takes this distribution as evidence that mechanisms for reducing the chromosome numbers have been far more efficient than ones leading to increases in chromosome number above n = 31. The distribution of chromosome numbers in moths is similar to that in butterflies (ROBINSON 1971). Trichoptera, the sister order of lepidopterans, is characterized by n = 30 (SUOMALAINEN 1969). There was one exception: FEDERLEY (1938) showed that among butterflies the family Lycaenidae (bluewings) has a modal number n = 24, different from that of all other lepidopterans.

Lepidopterans are, however, not an easy object for chromosome research. The chromosomes are small and lack primary constrictions. At the pachytene stage of meiosis the chromosomes are much longer than the mitotic ones and display a specific chromomere pattern (TRAUT 1976). As a consequence, progress in lepidopteran chromosome research has been slow (YOSHIDO et al. 2005a). About 40% of the chromosome surface in species with n = around 30 is covered with kinetochore plates (GASSNER

and KLEMETSON 1974; LUKHTANOV and DANTCHENKO 2002). Such nearly holokinetic chromosome structure should allow chromosome fragments to survive, since each fragment should be able to attach to the spindle. Likewise, translocations involving a fusion should almost always produce structures with a spindle attachment site. The telomeres have a role in the achiasmatic meiosis of lepidopteran females (REGO and MAREC 2003). In translocations involving a fusion telomeres have to be silenced in the new interstitial sites (YOSHIDO et al. 2005b).

The diversity of butterflies in South America is greater than in any other biogeographical region. About 40% of the described species of the superorder Papilionoidea (butterflies and skippers) are found in the Neotropics (LAMAS 2004). In addition, Hedyloidea, the sister group of Papilionoidea, is exclusively South and Central American (WAHLBERG et al. 2005). Important evolutionary concepts like mimicry were discovered and developed in South America by BATES and MÜLLER. Preventing the loss of this tropical biodiversity in the face of human population pressure (BROWN and BROWN 1991; BROWN 2005) has become the focus of an international multidisciplinary conservation effort. Butterflies have emerged as the flagship of invertebrate conservation. They have also secured their role as model systems in the study of ecology and evolution (HANSKI 2003; WATT and BOGGS 2003). Lepidopterans have been used as models in both population genetics (FISHER 1930) and cytogenetics (FEDERLEY 1913).

We publish here chromosome numbers for Neotropical Hesperiidae and Pieridae. This represents the final part of an extensive project. The author BROWN started in the late 1960s a broad survey of chromosome numbers of Neotropical butterflies, first in collaboration with Dr. H. DE LESSE of the Muséum national d'Histoire Naturelle of Paris, France, and then with Drs. ESKO SUOMALAINEN and BARBARA VON SCHOULTZ of the University of Helsinki, Finland, and Drs. T. EMMEL and P. ELIAZAR of the Museum of Florida at Gainesville. The project has resulted in a series of publications (started with DE LESSE and BROWN 1971), on Heliconiini (SUOMALAINEN and BROWN 1984; BROWN et al. 1992), on Papilionidae (EMMEL et al. 1995), on Ithomiini and Danaini (BROWN et al. 2004), Charaxinae and Satyrinae, i.e. bait-attracted Nymphalidae (BROWN et al. 2007a), Riodinidae and Lycaenidae (BROWN et al. 2012) and other Nymphalidae, in particular Biblidinae (BROWN et al. 2007b). The material comes from the entire Neotropical area and all major groups of butterflies are represented among some 3500 samples that cover about 1400 species and subspecies.

Here we consider the following questions: 1) is there evidence that $n = 31$ is the ancestral number among butterflies, from which other numbers are derived? 2) Why are numbers above $n = 31$ rare and below it relatively common? 3) How many modal numbers are there, and does their distribution in the phylogeny suggest that they represent independent events of differentiation? 4) Is there any evidence that the selective pressures associated with adaptation (in particular mimicry) would be reflected in the rate of karyotype evolution? 5) Why do South American groups of butterflies have the tendency to deviate from the lepidopteran modal numbers?

MATERIAL AND METHODS

KEITH BROWN collected the butterflies in the field throughout tropical America (or they were rarely reared in the laboratory). He fixed the gonads from male butterflies as described e.g. by EMMEL (1969) and BROWN et al. (1992). The fixed gonads were sent to the laboratories in Paris, Helsinki or Gainesville for further processing. In Paris and Helsinki the paraffin block sectioning method was used, while the laboratory at Gainesville used squash preparations. BARBARA VON SCHOULTZ did the practical laboratory work in Helsinki in the 1980s up to the year 1994. The late Dr. ESKO SUOMALAINEN of the Dept of Genetics of the Univ. of Helsinki checked the chromosome number counts. The reader may consult LORKOVIĆ (1990) for details on the differences between the two methods. We have also included the published data from MAEKI and REMINGTON (1960a, 1960b, 1960c), DE LESSE (1967, 1970a, 1970b; DE LESSE and BROWN 1971) made

with sectioning and WESLEY and EMMEL (1975) made with the squash method. ANJA and ANSSI SAURA went through the notes of Dr. BROWN at Campinas and checked them against the material at Helsinki. In collaboration with Dr. BROWN they put the manuscript together.

The Hesperiidae and Pieridae results given here are in part new, but include also results published earlier. Some chromosome numbers given here could not be reliably assigned to species. In all cases the voucher codes are given. The original laboratory notebooks and chromosome slides of the material are at the Finnish Museum of Natural History, Univ. of Helsinki, Finland. The material for the other families has been published earlier as parts of the series (see Introduction). Amounts of data for each taxon are variable. While Ithomiini (BROWN et al. 2004) and Heliconiini (BROWN et al. 1992) are exhaustively covered, we have less material for the rest. In each case, however, we feel that there is enough material to give a tolerably reliable picture of the chromosome numbers. Each species and subspecies (if variable) received a separate chromosome number. If the number is uncertain in closely related forms (that is, there are two or three alternatives), we have chosen the most common alternative. Chromosome numbers are given separately when they come from different individuals.

RESULTS AND DISCUSSION

Hesperiidae

Hesperiidae (skippers) have been usually considered the sister group of true butterflies (VANE-WRIGHT 2003; WAHLBERG et al. 2005, but see MUTANEN et al. 2010). Skipper diversity is highest in the Neotropics. More than 2300 out of the total of some 3700 species are Neotropical (HEPPNER 1991; MIELKE 2004). Table 1 gives the chromosome numbers of 75 Neotropical skippers. The Pyrrhopygini (former Pyrrhopyginae) is an endemic Neotropical clade nested inside the Pyrginae (WAHLBERG et al. 2005; WARREN et al. 2009). They have a peak at $n = 28$. The Eudaminae and Pyrgini have a sharp peak at $n = 31$, while the Hesperiinae have $n = 29$ as the most common number. This distribution of numbers agrees with the one published for the latter three subfamilies in the rest of the world (ROBINSON 1971; EMMEL and TREW 1973; LORKOVIĆ 1990). FREEMAN (1969) has given the chromosome numbers for "Megathyminae" (now a group within Hesperiinae, WARREN et al. 2009). They represent a southwestern Nearctic *Yucca*-feeding faunal element, with some representatives found in the margins of the Neotropical region. They have a major peak at $n = 26–27$ and a minor one at $n = 9–10$; *Agathymus aryxna* has $n = 5$. The overall evidence indicates that chromosome numbers of Eudaminae, Pyrginae and Hesperiinae conform to the lepidopteran

Table 1. *Haploid chromosome numbers for species and recognized additional subspecies of South American Hesperiidae and Pieridae. A comma between chromosome numbers indicates different individuals, a dash indicates uncertain alternative numbers within an individual. A voucher code is given for each new chromosome number. The identity of species which is uncertain is marked as "sp". Localities are grouped by region; lower case letters in parentheses indicate previous work (a = DE LESSE 1967, b = DE LESSE 1970a, c = DE LESSE 1970b, d = DE LESSE and BROWN 1971, e = MAEKI and REMINGTON 1960a, f = WESLEY and EMMEL 1975). Locality codes: AM = Amazonas (northwestern Brazil), CC = Chocó (western Colombia), DF = Brasília (central Brazil), EE = eastern Ecuador, ES = Espírito Santo (eastern Brazil), GO = Goiás (central Brazil), MG = Minas Gerais (central Brazil), MT = Mato Grosso (central Brazil), PA = Pará (northern Brazil), PE = Pernambuco (northeastern Brazil), RG = Aragua (northern Venezuela), RJ = Rio de Janeiro (southeastern Brazil), RO = Rondônia (western Brazil), SP = São Paulo (southeastern Brazil), VC = Valle de Cauca (western Colombia), WE = western Ecuador.*

Genus	Species	n =	No. studied pop./ind.	Locality
Family HESPERIIDAE				
Subfamily Pyrginae				
Tribe Pyrrhopygini				
Elbella	*lamprus*	40 (39 + 1 s)	1/1	DF(d)
Jemadia?	sp. (blue/white) 1261	32?	1/1	RO
Mimoniades	*montana* 1574	27	1/3	SP
	nurscia	28	1/1	Ecuador(a)
	nurscia malis Hesp 3	28	1/2	CC
	versicolor	28	1/1	DF(d)
	sp. 948	21	1/1	VC
	sp. 948	28	1/1	VC
Pyrrhopyge	*charybdis* 1571	14?	1/1	SP
	pelota	28	1/1	Argentina(a)
	sp. (UNH white at base) 1279	15	1/1	RO
Sarbia	sp. (narrow black on HW) 1583	30	1/1	SP
Tribe Pyrgini				
Achlyodes	*pallida (selva)*	15	1/1, 1/1	Bolivia(a), Mexico(b)
Anisocharia	*sublimbata*	31	1/1	Argentina(a)
Antigonus	*erosus*	31	1/1	Mexico(b)
	liborius	31	1/1	Argentina(a)
Chiomara	*asychis georgina*	31	1/1	Mexico(b)
	sp.	31	1/1	Trinidad(f)
Ebrietas	*anacreon*	31	1/1	Argentina(a)
	osyris	31	1/1	Argentina(a)
Erynnis	*funeralis (E. zarucco f.)*	31	1/2	Argentina(a)
Gesta	*gesta*	32	1/1	Tobago(f)
Grais	*stigmaticus*	31	1/2	Mexico(e)
Heliopetes	*arsalte*	30	1/1, 1/1	Bolivia(a), Mexico(b)
	omrina	30	1/1	Argentina(a)
Heliopyrgus	*americanus (Pyrgus a.)*	30	1/1	Chile(a)
Oechydrus	*chersis*	31	1/1	Bolivia(a)
Paches	*l. loxus (P. l. zonula)*	31	1/2	Guatemala(b)
Pyrgus	*bocchoris*	30	1/2	Argentina(a)
	fides	30	1/1	Chile(a)
Theagenes	*albiplaga*	31	1/2	Bolivia(a)
Trina	*g. geometrina*	31	1/1	RJ(d)
Zera	*z. zera*	34	1/1	RJ(d)
Subfamily Eudaminae				
Achalarus	*toxeus*	16 (15 + 1 s)	1/1	Mexico(e)
Astraptes	*anaphus*	31	1/1	Bolivia(a)
	naxos 1563	31	1/1	SP
	phalaecus	25	1/1	Guatemala(b)

(*Continued*)

Table 1. (*Continued*)

Genus	Species	n =	No. studied pop./ind.	Locality
Chioides	*catillus*	31	1/1	Mexico(e)
	albofasciatus (*C. catillus albofasciata*)	31	1/1	Mexico(b)
Entheus	*priassus pralina* 602	22	1/1	PE
Epargyreus	*barisses*	31	1/4	Argentina(a)
	clavicornis tenda	ca 29–30	1/1	Guatemala(b)
Phocides	*polybius phanias* 662	16	1/2	RJ
Tarsoctenus	*praecia plutia* Hesp 1	15	1/2	AM
Udranomia	*spitzi*	29	1/1	DF(d)
Urbanus	*d. dorantes*	31	1/1	Mexico(b)
	proteus	31	1/1, 1/1	Bolivia(a), Mexico(b)
	simplicius	31	1/1	Argentina(a)
	teleus	31	1/2, 1/1	Argentina(a), Bolivia(a)

Subfamily Heteropterinae

Butleria	*quilla*	29	1/1	Chile(a)

Subfamily Hesperiinae

Alera	*vulpina*	ca 27	1/1	Ecuador(a)
Arotis	*derasa (Euphyes d.)*	28	1/1	RJ(d)
Cymaenes	sp.	31	1/1	Tobago(f)
Cynea	*iquita*	29	1/1	Argentina(a)
Ebusus	*ebusus*	29	1/1	Mexico(b)
Euphyes	*leptosema*	ca 28	1/1	Argentina(a)
Hylephila	*fasciolata*	29	1/1	Argentina(a)
	phyleus	29	1/1	Argentia(a)
	signata	29	1/1	Chile(a)
Lychnuchus	*celsus*	30	1/1	RJ(d)
Polites	*vibex catilina*	29	1/2	Argentina(a)
Thargella	*caura*	25	1/1	RJ(d)
Vettius	*coryna*	31, ca 32	1/2	Ecuador(a)
	phyllus prona	26	1/1	RJ(d)
Wallengrenia	*premnas*	27	1/1	Argentina(a)

Family PIERIDAE
Subfamily Dismorphiinae

Dismorphia	*amphione astynome* 191	31	1/1, 1/1	GO, MG
	a. broomeae 823	30	1/1	RG
	a. praxinoe	30	1/1	Mexico(b)
	astyocha 325	16	1/1	ES
	c. crisia 134, *c. crisia* (*D. critomedia*)	13, 31	1/1, 1/1	RG, Bolivia(a)
	crisia foedora (D. critomedia foedora)	31	1/1	Colombia(a)
	hyposticta	48	1/1	Colombia(a)
	spio Pr 4	30	1/3	Puerto Rico
	thermesia 161, *thermesia*	19, 31	1/2, 1/1	RJ, Df(d)
	theucharila	18	1/1	Colombia(a)
	theucharila vitrea (*theonoë melanina*)	ca 14 + 1 s	1/1	Guyane(c)
	theucharila? ssp. M 2, Pr 1	26	1/1, 1/1	PA, RO
	zathoe demeter 971	31	1/1	WE
	sp. (like *Oleria zelica*) 1393	21	1/2	WE
	sp. (yellow) 959	22	1/1	WE
	sp. (yellow) 959	24	1/1	WE

(*Continued*)

Table 1. (*Continued*)

Subfamily Dismorphiinae				
	sp. (like *Oleria zelica*) 1523	24	1/1	WE
	sp. 1090	30–31	1/3	EE
	sp. (yellow) M 1	31	1/2	PA
Enantia	jethys (*Dismorphia j.*)	31	1/6	Mexico(b)
	lina psamathe (*Dismorphia p.*)	23, 24	1/1, 1/5	Argentina(a)
	lina galanthis 1168, 523	30	1/2, 1/2	GO, MT
	sp. nr *melite* 1473	31	1/1	EE
Lieinix	nemesis (*Dismorphia n.*)	31	1/10	Bolivia(a)
Moschoneura	pinthous (*Dismorphia p.*)	17	1/1	Guyane(c)
	sp. PI 8	15	1/1	VC
Patia	orise M 3	53	1/1	PA
Pseudopieris	nehemia	23	1/3	Argentina(a)
	viridula (*P. nehemia v.*)	23	1/1	Ecuador(a)
Subfamily Coliadinae				
Abaeis	nicippe (*Eurema n.*)	31	1/1, 1/2	Mexico(b), Mexico(e)
Anteos	clorinde	31	1/2, 1/5	Colombia(a), Mexico(e)
Aphrissa	statira (*Phoebis s.*)	31	1/2	Colombia(a)
Colias	dimera	31	1/4	Colombia(a)
	euxanthe hermina (*C. hermina*)	31	1/3	Peru(a)
	lesbia	31, 32	1/3, 1/1	Argentina(a), Ecuador(a)
	lesbia vautherii (*C. vautherii*)	31	1/2	Chile(a)
Eurema	albula	ca 28, 29	1/1, 1/1	Argentina(a), Colombia(a)
	arbela boisduvaliana (*E. boisduvaliana*)	31	1/1	Mexico(b)
	arbela graduata (*E. graduata*)	31	1/2	Bolivia(a)
	arbela gratiosa (*E. gratiosa*)	31	1/1	Colombia(a)
	daira	31	1/1, 1/1	Mexico(b)
	deva	31	1/2	Argentina(a)
	elathea platescens (*E. plataea*)	ca 31	1/1	Argentina(a)
	mexicana or *E. boisduvaliana*	31	1/1	Mexico(e)
	phiale	31	1/1	Bolivia(a)
	reticulata	31	1/2	Ecuador(a)
	salome	31	1/2	Bolivia(a)
	xantochlora	31	1/1	Ecuador(a)
Kricogonia	lyside	31	1/7	Mexico(e)
Nathalis	iole	31	1/1	Mexico(b)
Phoebis	argante	31	1/1, 1/1	Argentina(a), Ecuador(a)
	n. neocypris (*P. cipris*)	31	1/3, 1/1	Argentina(a)
	philea	31	1/1, 1/7	Mexico(b), Mexico(e)
	sennae	31	1/3	Trinidad(f)
	s. sennae (*P. eubule*)	31	1/1, 1/4	Bolivia(a), Colombia(a)
Pyrisitia	dina (*Eurema leuce d.*)	ca 31	1/1	Argentina(a)
	dina westwoodi (*Eurema calceolaria*)	31	1/1, 1/1	Guatemala(b), Mexico(b)
	leuce (*Eurema l.*)	31	1/1	Tobago(f)
	nise (*Eurema n.*)	31	1/2	Bolivia(a)
	nise nelphe (*Eurema n. n.*)	31	1/1	Guatemala(b)
	proterpia	31	1/3, 1/2	Colmbia(a), Mexico(e)
	venusta (*Eurema v.*)	30	1/1	Trinidad(f)
	v. venusta (*Eurema limbia*)	31–32	1/2	Bolivia(a)

(*Continued*)

Table 1. (*Continued*)

Subfamily Coliadinae				
Rhabdodryas	*trite (Phoebis t.)*	31	1/1	Argentina(a)
Teriocolias	*zelia (Eurema z.)*	31	1/3	Argentina(a)
Zerene	*cesonia*	31	1/1	Mexico(e)
Subfamily Pierinae				
Tribe Anthocharidini				
Cunizza	*hirlanda* Pi 7, Pr 6	15	1/1, 1/1	ES
	hirlanda ssp. 1277	15	1/1	RO
Hesperocharis	*costaricensis*	15	1/1	Mexico(b)
	erota 1572	16	1/1	SP
	marchalii	15	1/2	Bolivia(a)
Tribe Pierini				
Archonias	*brassolis negrina (A. bellona hyrnetho)*	25	1/1	Bolivia(a)
	brassolis rosacea (A. tereas r.)	26	1/1	Ecuador(a)
	brassolis tereas (A. tereas)	25	1/1	DF(d)
Ascia	*monuste*	27	1/4, 1/2	Argentina(a), Mexico(e)
	monuste suasa	27	1/2	Bolivia(a)
Catasticta	*ctemene alma (C. albina)*	25	1/1	Bolivia(a)
	flisa	25, 26–28	1/1, 1/3	Bolivia(a), Ecuador(a)
	pieris	25	1/3	Bolivia(a)
	reducta	29–31, ca 31	1/1, 1/1	Ecuador(a), Bolivia(a)
Glutophrissa	*drusilla (Appias d.)*	32	1/2, 1/6	Argentina(a), Mexico(e)
Hypsophila	*microdice (Tatochila m. m.)*	28	1/1	Argentina(a)
Itaballia	*demophile centralis*	25	1/1	Mexico(b)
Leptophobia	*aripa*	ca 26, 26	1/1, 1/1	Bolivia(a), Ecuador(a)
	eleone	26	1/2, 1/4	Ecuador(a), Bolivia(a)
	eleusis	26	1/1	Ecuador(a)
	philoma pastaza (L. subargentea pastaza, L. philoma)	26	1/1	Ecuador(a)
	tovaria	26	1/4	Ecuador(a)
Melete	*leucanthe*	24	1/1	Ecuador(a)
Melete	*lycimnia* 1374	24	1/1	EE
	lycimnia paulista	23	1/2	MG(d)
Pieriballia	*viardi*	26	1/1	Guatemala(b)
	viardi tithoreides (Itaballia tithoreides)	26	1/1	Ecuador(a)
Pereute	*swainsoni*	ca 26–27	1/1	MG(d)
Perrhybris	*pamela eieidias*	27, 28–29	1/1, 1/2	RJ(d)
	pamela flava Pie 10	28	1/2	ES
Perrhybris (?)	sp. yellow 1521	26 (?)	1/1	WE
Tatochila	*autodice*	28	1/1, 1/1	Argentina(a), Bolivia(a)
	mercedis	28	1/2	Argentina(b)
	mercedis arctodice (microdice a.)	28	1/1	Ecuador(a)
	orthodice (or *stigmadice*)	27 (or 28)	1/1 (or 1/3)	Argentina(a)
	sagittata	27	1/1	Ecuador(a)
	stigmadice (or *orthodice*)	28 (or 27)	1/3 (or 1/1)	Argentina(a)
	theodice	27	1/5	Chile(a)

modal of n = 29–31, while the Pyrrhopygini and "Megathyminae" represent a derived condition.

Papilionidae

On the basis of the fossil record, geographical distribution and phylogeny the swallowtail butterflies (Papilionidae) have been proposed to have originated in the East Palearctic or West Nearctic (Scriber 1995; Condamine et al. 2012). There seems to be a consensus (Vane-Wright 2003; Wahlberg et al. 2005; Mutanen et al. 2010) that the papilionids are the sister group of all other true butterflies. Papilionidae differ from most other groups of lepidopterans in that they have modal number n = 30. In the Neotropics this family is represented only by the subfamily Papilioninae. Emmel et al. (1995) and Brown et al. (1995) have shown that most (41 out of 65 species and subspecies) neotropical papilionids have n = 30. There is some variation, either slight, up to three numbers up or down from n = 30, or extensive, about 15 chromosomes up or down. The variation in chromosome numbers is not associated with either the phylogeny or larval food plants of the group. Maeki and Ae (covered in Ae 1995; Emmel et al. 1995) have made a very extensive series of crosses among *Papilio* species. They have shown that the degree of phylogenetic divergence between papilionid taxa has a linear correlation with the pairing of chromosomes at meiosis. In other words, the closer the taxa are, the more extensively their chromosomes pair.

Pieridae

Pieridae include the white and yellow butterflies found on all continents. The Neotropical region has by far the highest diversity. More than 70% of the Neotropical fauna is endemic. The family evidently originates from western Gondwana (Braby et al. 2006). There are four subfamilies, Coliadinae (sister to the rest), Pierinae, the almost exclusively Neotropical Dismorphiinae and the monotypic African Pseudopontinae. The latter two groups are closely related (Wahlberg et al. 2005). In South America many pierids mimic unpalatable Heliconiini and Ithomiinae while many are unpalatable of their own right. The mimics (e.g. the genus *Dismorphia*) may deviate widely from the general white-yellow pattern that otherwise characterizes the family. Virtually all Neotropical members (35 out of the total of 37) of the subfamily Coliadinae have n = 31. Coliadine chromosome numbers are rather uniform at about n = 31 in other parts of the world as well (Robinson 1971), with the African *Eurema birgitta* having n = 12 (de Lesse and Condamin 1962). Neotropical representatives of the two tribes of Pierinae − Anthocharidini and Pierini − have radically different chromosome numbers with no overlap. The Neotropical Anthocharidini are fixed at n = 15,

while Pierini have a peak at n = 26, and another at n = 28 (the genera *Perrhybris* and *Tatochila*) with a total range of n = 23 to n = 32. In other parts of the world the Anthocharidini are nearly fixed at n = 31 with n = 24 the lowest number observed (Robinson 1971). Pierini in other parts of the world have a peak at n = 25–26 (with about 20 species in each, Lorković 1990); again a single African species, *Leptosia alcesta*, has n = 12 (de Lesse and Condamin 1962; de Lesse 1968). Dismorphiinae have a peak at n = 31, with chromosome numbers ranging from n = 13 to n = 53. *Leptidea*, the single Palearctic genus of Dismorphiini, has a range from n = 28 up to n = 104 (Robinson 1971), unique among the Pieridae. Lukhtanov (1991) has reviewed the karyotype evolution of Pieridae worldwide.

Lycaenidae

Lycaenidae include the blue, copper, metalmark and hairstreak butterflies. In South America they are represented as the subfamilies Polyommatinae and Theclinae. A modest sample of 17 Neotropical species has a peak at n = 24 (Brown et al. 2012). As noted in the Introduction, the Lycaenids have been known to have a "type number" different from other lepidopterans as the numbers n = 23 and n = 24 are common. In his Fig. 5 White (1978, p. 73) has given chromosome numbers for the superfamily Lycaenoidea (families Lycaenidae and Riodinidae). There is, indeed, a peak at n = 23 with 16 species and a higher one at n = 24 with 46 species. White (1978) writes further that "spectacular increases and decreases of chromosome number have occurred in certain lineages of this family". That is, within the genera *Agrodiaetus* and *Lysandra*, the numbers range from n = 10 to n = 223. According to White (1978), the latter may well be the highest haploid number for any sexually reproducing metazoan. de Lesse (Brown et al. 2012) has shown that the transition from one chromosome number to another is orderly, geographically and taxonomically differentiated through southern Europe and Asia Minor.

Speciation accompanied with karyotype change has evolved three times independently in the related genera *Agrodiaetus, Lysandra* and *Plebicula* (Kandul et al. 2004). Whenever sympatric species of *Agrodiaetus* share the same chromosome number, they always show substantial morphological differentiation (Wiemers 2003). Lukhtanov et al. (2005) and Kandul et al. (2007) have shown that karyotype changes give rise to reinforcement when forms differing in chromosome number meet. Gompert et al. (2006) describe a case of introgression in the genus *Lycaoides*, where two species, each with n = 24, have met in an extreme habitat. The hybrids have evolved into an evidently reproductively isolated form with n = 24.

Riodinidae

Riodinidae are a mainly Neotropical family (about 1200 species in South America out of a world total of some 1300 species) of butterflies. They are often treated as a subfamily of Lycaenidae, but e.g. Vane-Wright (2003) and Wahlberg et al. (2005) have, in our opinion convincingly, shown that they represent the sister group of lycaenids. We have given the chromosome numbers for about 173 species and subspecies of Neotropical Riodinidae (Brown et al. 2012). In contrast to lycaenids, which show remarkably stable chromosome numbers in our limited Neotropical sample, the riodinids are characterized with extensive karyotypic instability across the family. There is very little evidence for any relationship with the lycaenids.

The riodinid chromosome numbers range from n = 9 up to n = 110, although numbers above 31 are rare. The subfamily Euselasiinae is characterized with n = 29 as the most common chromosome number, but the tribes and subtribes of the derived subfamily Riodininae have quite divergent distributions of chromosome numbers with little evidence of a modal number. We (Brown et al. 2012) argue that the karyotypic instability of riodinids may be related to population structure, characterized by small fragmented populations with little migration. Such a population structure can give rise to rapid speciation and karyotype changes may accumulate.

Nymphalidae

Nymphalidae are the largest family of butterflies with over 6500 species worldwide. Some 3000 species are found in the Neotropics. The tribes Ithomiini, Brassolini, Morphini and Heliconiini are exclusively Neotropical. The subfamily Libytheinae, sister group of all remaining nymphalids, has four Neotropical species (Kawahara 2006). Two subspecies of *Libytheana carinenta* had n = 31–33 (Brown et al. 2007b).

Danaini, the sister group of Ithomiini, are characterized by n = 30 with little variation (Brown et al. 2004). They are also known models for mimics worldwide. The Ithomiini are (arguably) an exclusively Neotropical group with about 300 species (Heppner 1991). Brown et al. (2004) give chromosome numbers for more than a thousand populations of 242 species. Ithomiini are prime movers for mimicry rings (Brown et al. 2004). Their chromosome numbers range almost continuously from n = 5 to about n = 120, with modal values at n = 12–18. In addition to the main modal number of n = 14 there is a more modest peak at n = 31. Evidently the chromosome set of about n = 31 of Ithomiini has been halved early in their history through extensive chromosome fusions. This event has been followed through differentiation in each genus and tribe. At least 17 species in 15 genera show stable karyotypes over much of the Neotropics, while at least 40 species show extensive geographical variation in number. There is no good evidence that this variation would be accompanied by reduction in fertility or incipient speciation. These butterflies are common and highly gregarious; they may also be migratory as a community.

The tribes Brassolini with n = 29 and Morphini with n = 28 have stable modal numbers (Brown et al. 2007a). The most common chromosome numbers of Neotropical Satyrinae are n = 29, n = 25 and n = 13 with a rather even distribution among the numbers extending to a low n = 6 (Brown et al. 2007a). The genus *Pierella* of the tribe Haeterini has many species with n = 29. All five genera of the first clade (Peña et al. 2006) of the subtribe Pronophilina are fixed for n = 29, while genera of the second clade have variable numbers. The numbers for Euptychiini range from n = 6 up to n = 105, with many at n = 12–18 and n = 21–30 but few above n = 30. There is what appears to be variation within species; a phenomenon most easily seen in the genus *Taygetis*. Already de Lesse (1967) and Wesley and Emmel (1975) noted within species variation in Euptychiini. Some of this variation is attributable to chromosome fragmentation but the presence of cryptic sibling species cannot be excluded as a plausible explanation.

Lorković (1990) noted, on the basis of less extensive material, that the chromosome numbers of Neotropical Satyrini clustered rather weakly around n = 29. With the exception of the Holarctic genus *Erebia* that had variable chromosome numbers, Satyrini elsewhere have a distinct modal number n = 29. The genus *Erebia* has been studied very extensively by Lorković and de Lesse (Lorković 1990; Brown et al. 2007a). Chromosome number change is a very orderly part of the speciation process in *Erebia*. Species differ chromosomally from each other: they are largely allopatric and show good evidence for prezygotic isolation in addition to the postzygotic one conferred by differing chromosome numbers (Lorković 1958).

The two tribes, Anaeini and Preponini, of Neotropical Charaxinae have quite different distributions of chromosome numbers (Brown et al. 2007a). While the Anaeini (with the exception of the genus *Hypna*) have in general rather high numbers, with many n = 31, the Preponini range from n = 9 to n = 19, with one exception.

Brown et al. (2007b) give the chromosome numbers of about 80 species or subspecies of Neotropical Biblidinae. There is some variation in numbers but n = 31 is, nevertheless, the most common chromosome number in Biblidinae. The seven species of Neotropical Apaturinae covered by Brown et al. (2007b) have all n = 31 or one or

two steps up from it. The few Neotropical species of Cyrestinae have also n = 32 (Brown et al. 2007b).

Wahlberg et al. (2005) place the Limenitidinae as the sister group of Heliconiinae. Limenitidinae have n = 30 as the modal number not only in the Neotropics (Brown et al. 2007b) but also elsewhere (Robinson 1971). The few Neotropical species of the tribes Argynnini and Acraeini covered by Brown et al. (2007b) have n = 31. There are two exceptions but they may be due to methodological error (Brown et al. 2007b).

Two papers (Suomalainen and Brown 1984; Brown et al. 1992) covered the tribe Heliconiini. In the genus *Philaethria*, at least one species, *P. dido*, turned out to be a complex with several widely divergent chromosome numbers, ranging from n = 12 up to n = 88 (Suomalainen and Brown 1984). There is no evidence for hybridization between these forms and they apparently constitute *bona species.* Another genus, *Podotricha*, has two closely related rare species with n = 9 and n = 28; they mimic some common Heliconiini with n = 31 or n = 21. Many genera of Heliconiini have n = 31, with *Neruda* going down to n = 21 and *Heliconius* stabilized at n = 21. We argue, on the basis of chromosome behavior seen in natural hybrids between morphs and species and other evidence for among species hybridization (Gilbert 2003) that interspecies hybridization maintains the n = 21 stably within the genus *Heliconius* (Brown et al. 2007b). The most derived members of the genus again deviate from n = 21, going up to n = 56–57 and n = 59–62. With the exception of *H. hewitsoni* that has n = 21, they all are pupal mating species, i.e. forms in which the males mate with the female before she ecloses from the pupa. The female choice element of sexual selection is relaxed. Following the argument of Gilbert (2003) we proposed that the karyotypic instability seen in pupal mating *Heliconius* is evidence that sexual selection is a conservative force that stabilizes the karyotype (Brown et al. 2007b). Mavárez et al. (2006) proposed that *H. heurippa* has arisen as a hybrid between *H. cydno* and *H. melpomene*: the hybrids are reproductively isolated from the parent species. The chromosomes of hybrids between *Heliconius* species having the same chromosome number pair normally (Brown et al. 2007b) and there is good evidence for introgression, in particular at loci involved in mimicry (Dasmahapatra et al. 2012). The other tribes of Heliconiinae (Acraeini and Argynnini) show some deviation from n = 31 both in the Neotropics (Brown et al. 2007b) and in other parts of the world (Robinson 1971).

Almost all species of Neotropical Nymphalinae covered by Brown et al. (2007b) have n = 31. There are a couple of exceptions that have about half that number (n = 11 and n = 15). The Nymphalinae of other parts of the world have a strong modal n = 31 as well (Robinson 1971).

General discussion

The chromosome number material for the butterflies from Neotropical region covered here exceeds the material available from elsewhere. Very few Neotropical butterflies were included in the histogram of 740 species given by White (1978). In the Introduction section we posed five questions, the first of which was whether n = 31 represents the ancestral condition among Papilionoidea. The answer is without doubt positive. The numbers 29 and 30, and in most cases the lepidopteran modal of n = 31, are found in members of major taxa. There is extensive synteny among lepidopteran taxa (Dasmahapatra et al. 2012). Our second question: why numbers above n = 31 are uncommon deserves to be studied. There are species and genera that exceed n = 31 but no large group of butterflies does it. Lukhtanov et al. (2005) and Kandul et al. (2007) have developed an approach that may prove fruitful in answering the question; Yoshido et al. (2005a, 2005b) have shown that the position of telomeres involved in fusion and fragmentation can be determined with relative ease. We also asked how many modal numbers there are. As for the number of derived modal numbers, we have shown that there are many, from the low n = 14 and n = 15 that characterize Ithomiini and Anthocharidini to n = 21 of the genus *Heliconius* to n = 24 of the Lycaenidae through n = 26 of Pierini to n = 28 of Pyrrhopygini and Morphini to tribes and larger taxa with n = 29, 30 and 31. Finally, Riodinidae and Euptychiina among Satyrines do not seem to have any distinct modal number. Our fourth question was whether the selective pressures associated with adaptation (as exemplified by mimicry) would be reflected in the rate of karyotype evolution?

Factors underlying karyotype evolution: agents of change

We have tentatively identified factors that may drive karyotype evolution. *Mimicry* is a complex phenomenon that is prevalent in many Neotropical groups of butterflies. There appears to be a general tendency of groups involved in mimicry (e.g. Ithomiini, Riodinidae, many Charaxinae, Satyrinae etc.) to show karyotype instability. Mimicry causes a relentless and strong selection pressure that drives phenotypic differentiation. Chromosome number changes are probably accidental. Karyotypic change is most effective in small, isolated or semi-isolated populations (Faria and Navarro 2010). Differences in chromosome number reinforce speciation that may originally have arisen through other mechanisms. Kandul et al. (2007) have shown that chromosomal rearrangements are directly involved in the speciation of the lycaenid genus *Agrodiaetus*. We may note that Ithomiini and in particular Riodinidae are a case point. They are both mimetic and show extensive population structuring; at the same time

they show very evident karyotype instability. Host plant diversification is an additional factor that evidently has contributed to the diversification of phytophagous insects; JANZ et al. (2006) have shown that it is associated with the speciation of Neotropical Nymphalidae.

Stabilizing factors

Migration will counteract the effects of local differentiation. Coliadinae, many Nymphalidae and Lycaenidae are known migrants. They are characterized with stable or very stable chromosome numbers. *Hybridization* among related species should be relatively easy if all share the same chromosome numbers (MALLET 2007). Müllerian mimicry imposes a strong selection pressure on the genus *Heliconius*. GILBERT (2003) has shown how different morphs are maintained through a combination of selection and among species hybridization. All species involved share n = 21, and their chromosomes seem to pair regularly in species crosses. *Sexual selection* may be tentatively identified as a further factor stabilizing the chromosome number. GOMPERT et al. (2006), MAVÁREZ et al. (2006), BAXTER et al. (2010) and DASMAHAPATRA et al. (2012) show that *introgression* and *hybridization* between forms sharing the same chromosome number may be at least a potential factor explaining cases of stable chromosome numbers.

WHITE (1973) explained the stability of lepidopteran chromosome numbers through *karyotype orthoselection*. He argued that the continued reappearance of the same chromosome number in many lineages may not be evidence of common ancestry (a hypothesis that we have refuted above) but is rather evidence for an optimum karyotype that will be restored once it has been perturbed by the process of speciation. He assumed that factors associated with the dimensions of the spindle apparatus in dividing cells would be the agents of this selection. As we mentioned in the Introduction, telomeres are expected to be important in such a process. LUKHTANOV and DANTCHENKO (2002) have shown that a mechanism like the one envisioned by WHITE (1973) may operate in lepidopterans. PAPA et al. (2008) and DASMAHAPATRA et al. (2012) have shown that gene order is conserved between distantly related *Heliconius* species and, in addition, there is clear-cut synteny with *Bombyx mori*. Genome structure is evidently conserved among the higher Lepidoptera. We may also point out that a process that involves a concerted *fusion* of all chromosomes with half the modal chromosome number, i.e. n = 14–16 is certainly common and has arisen repeatedly in all groups of Neotropical butterflies. The resulting chromosomes are all of an equal size. The process has evidently been involved in the origin of groups like Ithomiini and Neotropical Anthocharidini. Extensive *fragmentation* results

again in chromosomes of equal size, albeit one pair (presumably the sex chromosomes) remains unaffected (SUOMALAINEN and BROWN 1984; BROWN et al. 2004). SEILER (1925) identified fragmentation as an agent of change in chromosome numbers in lepidopterans. The phenomenon was, in part, understood later when the nearly holokinetic structure of the chromosomes became known (BAUER 1967). Nevertheless, fusion and fission of holokinetic chromosomes restricts gene flow in sedges and this effect increases in proportion of chromosomal difference (HIPP et al. 2010).

Our last question was why do the Neotropical groups of butterflies have a tendency to deviate from the modal n = 29–31 that characterize the order Lepidoptera? The observation that many Neotropical groups have modal numbers different from the "type number" and that some do not have a modal number at all has emerged in the course of this study. Our database is much larger than any made earlier, and allows us to draw conclusions, some obvious, some tentative.

DOBZHANSKY (1950), with characteristic brilliance, pointed out that biotic interactions (such as mimicry, parasitism and predation) are the agents of selection in the tropics, while in harsher environments physical factors are of paramount importance. Our discussion is couched very much around this idea. Chromosome evolution has been long a rather neglected field in the study of Lepidoptera. We trust that its time will come and that the tools of phylogeny in combination with molecular cytology (cf. JORON et al. 2011; DASMAHAPATRA et al. 2012) will be used to throw light on the evolution of the richest biodiversity in the world, seen in South America.

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