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A male-biased primary sex ratio and larval mortality in *Eucheira socialis* (Lepidoptera: Pieridae)

Dessie L.A. Underwood* and Arthur M. Shapiro

Section of Evolution and Ecology, University of California, Davis, CA 95616, USA

ABSTRACT

We investigated the sex ratio and sex-biased mortality in the Mexican pierid butterfly, *Eucheira socialis westwoodi*. We studied two populations between 1990 and 1997 along Mexico Highway 40, which runs from Mazatlán, Sinaloa to Durango, Durango. Populations occurring between km 64 and 101 and between km 163 and 213 were designated 'eastern' and 'western', respectively. We determined the primary sex ratio of egg masses from these populations in 1992, 1995, 1996 and 1997. The primary sex ratio varied from 68.0 to 75.5% male and from 63.7 to 79.3% male in the eastern and western populations, respectively. The frequency of significantly male-biased full sib groups was between 55 and 100% in the east and between 67 and 94% in the west. For all years, in both populations, the primary sex ratio was significantly different from 1:1. If the probability of a given zygote being male is the same across sibships, the distribution of the primary sex ratio should be binomial. For years where at least 10 egg masses were collected, the distribution of primary sex ratio was significantly different from a binomial in all years in the east and two of three years in the west. We studied larval and pupal mortality in these populations in 1990, 1991 and 1997. Larval mortality was consistently disproportionately male, varying from 83 to 100% male. Pupal mortality tended to be female-biased. The operational sex ratio varied from 65 to 71% male in the east and from 70 to 76% male in the west.

Keywords: Lepidoptera, Mexico, Pieridae, sex-biased mortality, sex ratio.

INTRODUCTION

The evolution of the primary sex ratio is a fundamental and largely enigmatic aspect of natural history theory for many species. Sex ratio can influence the reproduction and fitness of nearly all individuals in a population. The probability of offspring finding a mate and the probability and degree of inbreeding are both potentially influenced by the sex ratio. Population parameters are also affected; for example, in species where males can mate with many females, the intrinsic rate of growth is largely dependent upon the number of viable females in the population. Inequalities in sex ratio should lead to inequalities in the reproductive values of sons and daughters and the subsequent evolution of an equal sex ratio (Fisher, 1930). In many species, the primary sex ratio is not 1:1 and theory on the evolutionary forces leading to and maintaining such biases has proved to be fertile ground

* Corresponding author: DLU, Biology, Calif. State U., Long Beach 90840, DLUNDERW@CSULB.EDU

(e.g. Hamilton, 1967; Charnov, 1982; Silk, 1984; Karlin and Lessard, 1986; Werren, 1987a; Wrensch and Ebberts, 1993).

Organisms with chromosomal sex determination should produce progeny in equal proportions of males and females simply due to segregation of the sex chromosomes during gamete production. Biased primary sex ratios can result from either meiotic drive or differential fate of the gamete types. Sex-linked meiotic drive occurs when there is an unequal proportion of X and Y (or O) carrying gametes produced by individuals that are the heterogametic sex. Theory predicts the fixation of meiotically driven genes. For populations harbouring meiotic drive systems involving the sex chromosomes, extinction can be avoided only by the evolution of suppressors and/or resistance genes, and/or by counterbalancing selection (Hamilton, 1967).

We report here that *Eucheira socialis westwoodi* (Lepidoptera: Pieridae), a diploid organism with chromosomal sex determination, exhibits an extremely male-biased primary sex ratio. The primary sex ratio is defined as the sex ratio at conception. As female butterflies are the heterogametic sex and female meiosis is arrested in metaphase I until after fertilization and oviposition, the sex ratio of hatchlings is the primary sex ratio. Lepidoptera hatchlings can be sexed using external morphology (Underwood, 1994b). The sex ratio bias reported here is unusual because the bias is towards males, the frequency of individuals producing biased progeny is great, and the bias is known to have persisted for six generations and in two geographically distinct populations. We discuss possible mechanisms producing this unusual bias and probable evolutionary factors involved in its maintenance.

METHODS

Study system

Eucheira socialis westwoodi is a pierid butterfly endemic to the Mexican highlands and found from northern Sonora to Jalisco (Hoffmann, 1910; Beutelspacher, 1983; Díaz Batres and Boudinot, 1986; Kevan and Bye, 1991; Underwood, 1994a). An adult female will typically mate once and oviposit one large mass of up to 400 eggs on the undersurface of a single leaf of madrone (*Arbutus* spp.: Ericaceae). Larvae are gregarious throughout all six instars and spin a hollow silken nest. There is one generation per year. Larvae do not diapause and pupation occurs within the nest with adult emergence between late May and the end of June. After adult emergence, nests are not reused by the next generation.

In the course of studying its ecology and host relations (Underwood, 1994a), we noted an apparently consistent sex-ratio bias throughout the life cycle of *E. s. westwoodi* which motivated the present study. Two populations of *E. s. westwoodi* were sampled along Highway 40, which runs between Mazatlán, Sinaloa and Durango City, Durango. Eastern and western populations were defined as those occurring between km 64 and 101 and between km 163 and 213, respectively. This division makes biological sense, as *Eucheira* does not occur along the road between approximately 110 and 160 km west of Durango City. Electrophoretic studies indicate that these populations are completely isolated (Porter *et al.*, 1997).

Egg masses are frequently oviposited near one another on the same terminal branches (Underwood, 1992). As larvae expand their nest, several sibships may coalesce to form one nest. Here, 'sibship' refers to a group of larvae which were known to be full sibs. 'Nest' refers to a group of larvae which may or may not be full sibs, but which inhabit a single nest.

Determination of primary sex ratio

Egg masses were collected in the field in June 1992, 1995, 1996 and 1997 and transported under permit to UC Davis in carry-on luggage (see Table 1 for sample sizes). Each egg mass was placed in a separate covered petri dish and monitored daily for hatching. Hatch date and number of eggs failing to hatch were recorded. Hatchlings were fed leaves of the California species of madrone, *Arbutus menziesii*. Leaves were kept fresh by placing petioles in dampened tissue paper and were changed as needed.

Larvae were sexed by examining the ventral surface of the 8th and 9th abdominal segments under a dissecting microscope with 20× oculars and 50× objective. Males have one central pit located on the 9th abdominal segment, while females exhibit two pairs, one each on the 8th and 9th segments (Underwood, 1994b). At least 40 first instar larvae per sibship were sexed in 1992 and at least 50 individuals per sibship were sexed in 1995–97.

Distributions of sex ratio for each year and site were tested against the hypothesis that the true sex ratio was 1 : 1 and binomial using replicated goodness-of-fit tests (G-Statistics).

Determination of sex-biased larval mortality

Many larvae fall behind their nest mates in development. For a given nest, there may be last instar larvae as well as pupae and eclosing butterflies present. The pupal stage lasts 3–4 weeks, meaning larvae alive at the time of adult emergence are at least 1 month behind their nest mates developmentally. These larvae presumably fail to pupate and are identified readily as they exhibit abnormal behaviour by remaining outside the nest during the day. Larvae exhibiting similar behaviour in the laboratory cease feeding and die without pupating. To determine whether there was a sex bias in larvae failing to pupate, larvae were taken from nests in the field during the flight seasons of 1990, 1991 and 1997, and placed in preservative. Larvae were sexed in Davis, California by dissection and examination of gonads or by external morphology as described above.

Twenty nests containing last instar larvae were collected at km 64 in March 1991 for use in another study (see Underwood, 1994a, for details on methodology). As larvae died, they were placed in preservative. All, or at least 40, larvae failing to pupate were sexed from each nest. The percent dead male larvae was compared to the total percent males for each nest using the Wilcoxon signed ranks test.

Determination of sex-biased pupal mortality and adult sex ratio

Eucheira larvae remain associated with their nest throughout all six larval instars and they pupate inside this larval nest. After adult emergence, each nest contains a perfect record of pupal fate. Adults successfully emerging leave an empty pupal case. Parasitized pupae are discoloured and are pierced with one or more parasitoid emergence holes. Some nests also contain dead pharate adults – fully formed butterflies that died at the end of their development and failed to eclose.

In June 1990, 1991 and 1997, we collected nests from the western and the eastern populations (see Table 3 for sample sizes). Three categories of pupal cases were used: empty pupal cases representing emerged butterflies, parasitized pupal cases and pharate adults. Pupal cases were categorized and sexed (see Scott, 1986, for diagrams on sex differences in pupae). Adult sex ratio was estimated by the sex ratio obtained from sexing empty pupal

cases. If there was no sex bias in pupal mortality, parasitization, or emerged adults, the sex ratio of these pupal cases should not differ from the sex ratio of all the pupae in a given nest. For nests with at least 10 pupae per category, we compared percent male and the three categories of pupae within a nest using Wilcoxon signed ranks tests.

To test for a trend across years, the individual Wilcoxon signed ranks tests were combined into a single test. Using the *P*-values for each of the individual tests, the statistic $X = -2\ \Sigma^{n}_{i=1}\ \ln(p_i)$ will have a χ^2 distribution with $2n$ degrees of freedom, where n is the number of individual tests (Sachs, 1982). For each population, the tests for the 3 years were combined in this manner.

Test for an effect of maternal age on sex ratio

Golanski (1968) induced male-biased sex ratios (60–68% male) in *Bombyx mori* by delaying fertilization by 3–8 days. We investigated delayed fertilization as a potential sex-ratio distorter in *E. s. westwoodi*. In the field, over 300 newly eclosed females were kept from males for 2–6 days. Each female was marked with identifying numbers on the wings and released. Forty-four females were later found ovipositing. These egg masses were collected, reared in the laboratory, and the primary sex ratio determined as described above. As adults are physically incapable of feeding, it is unlikely that females live much longer than 6 days under field conditions.

RESULTS

Table 1 presents a summary of the total number of egg masses collected and hatching success. Some sibships were used in other experiments and the primary sex ratio was not determined. Hatching success and its relationship to sibship sex ratio provides information regarding possible mechanisms producing the biased sex ratio and evolutionary factors in its maintenance. If egg hatch failure is positively related to sibship sex ratio, it suggests that embryo death may be sex-biased, as is commonly seen with cytoplasmic sex ratio distorters (see Hurst, 1993, for a review). Egg hatch failure and sex ratio were not related (Fig. 1).

Table 1. Summary of sample sizes of egg masses collected and hatching success

Population	Year	No. of egg masses collected	No. of egg masses scored for primary sex ratio	No. of egg masses with ≥90% hatch success	No. of egg masses with 0% hatch success
Eastern	1992	108	52	95	0
	1995	167	66	149	1
	1996	37	22	22	15
	1997	49	2	0	46
Western	1992	3	3	3	0
	1995	26	13	26	0
	1996	14	14	14	0
	1997	39	18	4	5

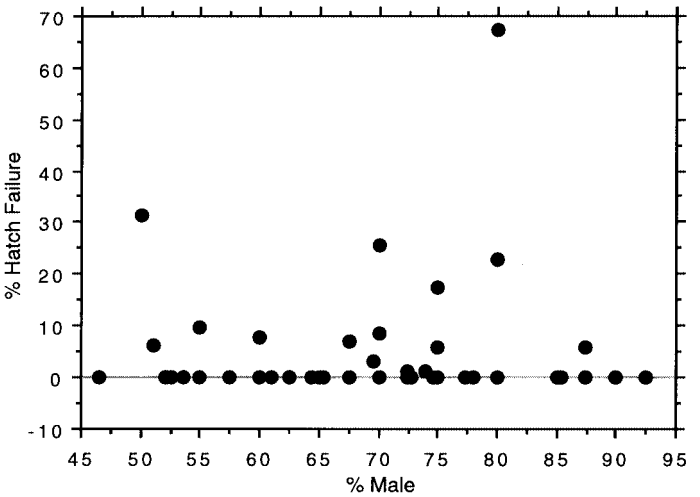

Fig. 1. Egg hatch failure versus sex ratio in one year in one population of *E. s. westwoodi*. There was no significant relationship in any other year in either population.

If the probability of a given egg being male is the same across sibships, the distribution of primary sex ratio should be binomial. The replicated goodness-of-fit test produces three values, G_H, G_P and G_T (Table 2). The G for heterogeneity (G_H) tests whether the sex ratios are homogeneous. For those years with sample sizes greater than 10, six of the seven year–population combinations were significantly different from a binomial distribution due to excessive variability around the mean. The pooled G (G_P) provides us with a measure of whether the pooled data deviate from expectation; in this case, from a 1 : 1 sex ratio. In both populations for all 4 years of the study, the primary sex ratio was significantly male-biased ($P < 0.001$). The frequency within each population of significantly male-biased sibships varied in the east from 55 to 100%; in the west it varied from 67 to 94%. Mean primary sex ratios for these same years varied from 68 to 76% male in the east and from 64 to 79% male in the west. Clutch size and primary sex ratio were unrelated (Fig. 2).

Table 3 summarizes the population level sex ratios of larvae failing to pupate, parasitized pupae, pharate adults and emerged adults. Larval mortality was consistently disproportionately male-biased (83–100% male) in both populations in all years for which larvae were collected. Parasitized pupae were relatively female-biased in five of six data sets. Mortality as pharate adults was more common in the east than in the west and tended to be relatively female-biased, although 1997 was a notable exception. Pupal mortality was marginally significantly female-biased ($P = 0.057$). The operational sex ratio, as implied by the sex ratio of emerged adults, varied in the east from 65 to 71% male and in the west from 70 to 76% male. In general, the primary sex ratio tended to be more male-biased than the adult sex ratio. The decline in sex ratio was due to male-biased larval mortality.

The sex ratio of larvae dying in the last instar from nests collected in March 1991 and reared in the laboratory was significantly more male-biased than the sex ratio of their natal nest (paired t-test, $P = 0.015$). Some of these nests suffered over 50% larval mortality, which changed the operational sex ratio of a nest by as much as 20%.

Table 2. Results of replicated goodness-of-fit (*G*-statistics) testing for a 1 : 1 primary sex ratio from two populations of *Eucheira socialis westwoodi* over 4 years

	1992	1995	1996	1997
Eastern population				
No. of sibships	52	66	22	2
% Male ($\bar{x} \pm$ S.D.)	71.1 ± 12.0	68.0 ± 12.3	67.9 ± 13.9	75.5 ± 4.0
No. of larvae sexed ($\bar{x} \pm$ S.D.)	55 ± 35	71 ± 47	49 ± 8	50 ± 0
No. of sibships with individual *G*-statistics with *P*-values of:				
$P \leq 0.01$	34	38	11	2
$0.01 < P \leq 0.025$	2	2	0	0
$0.025 < P \leq 0.05$	0	5	1	0
$P > 0.05$	16	23	10	0
Percent frequency of sibships significantly biased ($P \leq 0.05$)	69	68	55	100
G_H	195.07*	309.46*	87.61*	0.48
G_P	470.66*	617.27*	123.62*	26.16*
G_T	665.73*	926.73*	211.23*	26.64*
Western population				
No. of sibships	3	13	14	18
% Male ($\bar{x} \pm$ S.D.)	63.7 ± 8.8	75.2 ± 11.0	78.2 ± 7.4	79.3 ± 8.3
No. of larvae sexed ($\bar{x} \pm$ S.D.)	40 ± 0.6	50 ± 0	50 ± 6	50 ± 0
No. of sibships with individual *G*-statistics with *P*-values of:				
$P \leq 0.01$	1	11	13	16
$0.01 < P \leq 0.025$	1	0	0	1
$0.025 < P \leq 0.05$	0	1	0	0
$P > 0.05$	1	1	1	1
Percent frequency of sibships significantly biased ($P \leq 0.05$)	67	92	93	94
G_H	2.69	40.66*	20.66	35.12*
G_P	9.11*	173.37*	239.27*	330.55*
G_T	11.81*	214.02*	259.91*	365.67*

* $P < 0.001$. $G_H = G$ for heterogeneity; G_P = pooled G; G_T = total G.

The results of comparing the percent male dead larvae, parasitized pupae, pharate adults and emerged adults to the percent males of all pupae in a given nest are shown in Table 4. Although most year–population–class combinations have *P*-values greater than 0.05, the direction of the bias is consistent. Combining these individual tests for each class of pupa shows a clearer trend in relative female-biased death due to parasitization and becoming pharate. Biases in pupal mortality resulted in a disproportionately male-biased emerged adult sex ratio. These shifts in sex ratio, however, are minor in comparison to the decline in sex ratio from hatching to pupation. Female age and progeny sex ratio were unrelated (Fig. 3).

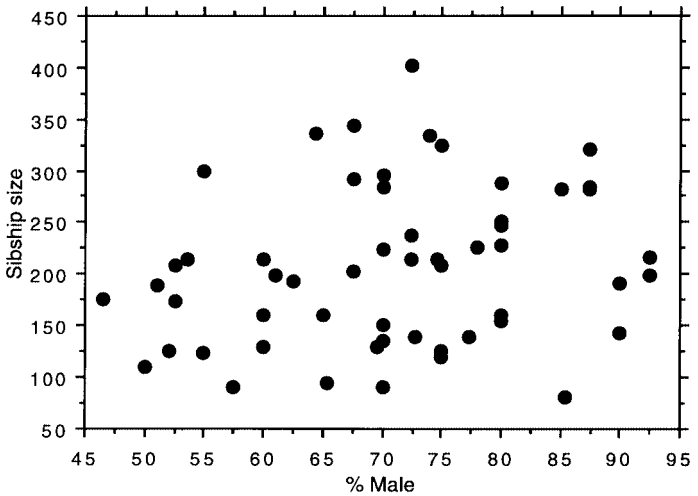

Fig. 2. Clutch size versus sex ratio in one year in one population of *E. s. westwoodi*. There was no significant relationship in any other year in either population.

Table 3. Percent males in the different life stages examined. Data from all nests are combined to give one population value for percent males[a]

	1990		1991		1997	
Life stage	*n*	% Male	*n*	% Male	*n*	% Male
Eastern population						
Dead larvae	40	90.0	45	100.0	100	91.0
All pupae	5072	65.0	5190	65.0	3737	69.0
Parasitized pupae	172	77.9	730	59.5	353	63.7
Pharate adults	261	55.6	817	65.7	612	61.3
Emerged adults	4639	65.1	3643	66.2	2772	71.4
Western population						
Dead larvae					31	83.9
All pupae	855	73.9	1661	66.5	1610	71.2
Parasitized pupae	339	69.9	877	64.1	669	67.9
Pharate adults	8	87.5	112	66.1	93	81.7
Emerged adults	508	76.4	672	69.8	848	72.8

[a] Data from nests excluded from the analyses (Table 4) because they contained fewer than 10 individuals in a class are included here. Sample sizes are total number of sexed individuals.

DISCUSSION

To understand the evolution and maintenance of a sex-ratio distorter, we need to examine both the proximate and the ultimate causes. The proximate mechanisms involve how sex is determined and how sex determination is inherited, whereas ultimate causation

Table 4. Results of Wilcoxon signed ranks tests comparing the sex ratios of pupae of different fates with the sex ratio of the entire nest[a]

	1990			1991			1997			Combined	
Life stage	*n*	Direction of bias	*P*	*n*	Direction of bias	*P*	*n*	Direction of bias	*P*	χ^2	*P*
Eastern population											
Parasitized pupae				17	Female	0.163	11	Female	0.284	6.1	0.189
Pharate adults	8	Female	0.069	26	Female	0.228	18	Female	0.122	12.5	0.051
Emerged adults	51	Male	0.790	80	Male	0.232	37	Male	0.002	15.8	0.015
Western population[b]											
Parasitized pupae	9	Female	0.021	18	Female	0.948	20	Female	0.093	12.6	0.050
Emerged adults	13	Male	0.311	20	Male	0.280	20	Male	0.100	9.5	0.148

[a] All comparisons were made within nests. Only nests for which there were at least 10 in a class were included. [b] No nests had more than 10 pharate adults in the western population.

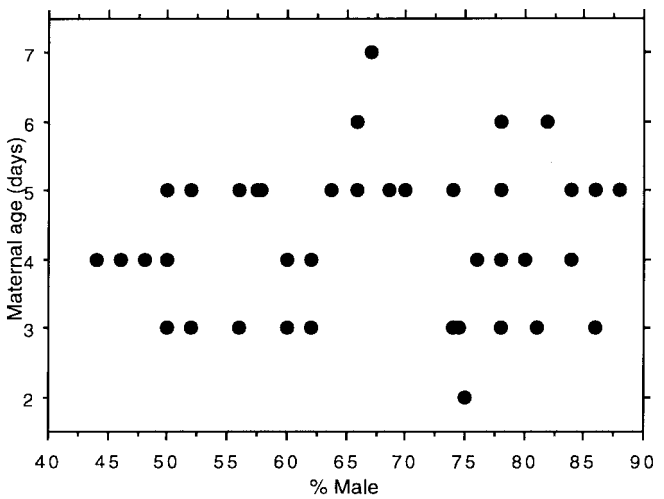

Fig. 3. Sex ratio as a function of mothers' ages.

encompasses all the selective forces acting on the population harbouring the distorter. The proximate mechanism sets limits on or defines the possible evolutionary pathways the system may take. For example, in haplodiploid species, where female behaviour (by releasing or not releasing sperm) determines the sex of progeny, evolution of adaptive facultative sex ratio adjustment may occur (Werren, 1987b). In diploid organisms with chromosomal sex determination, biased primary sex ratios are necessarily products of genetic conflict between the sex ratio distorter and the rest of the genome (Charnov, 1982; Hurst *et al.*, 1996).

Lepidoptera typically have XY/XX or X/XX sex chromosomes with heterogametic females. Variants with multiple copies of the X and/or the Y chromosomes have also been found (Nilsson *et al.*, 1988; Suomalainen, 1969). However, the sex-determining factors in Lepidoptera are largely unknown (Traut and Marec, 1996). It is assumed that X/XX sex chromosome systems consist of male-promoting factors on the X chromosome and female-promoting factors located either in the cytoplasm or on the autosomes. A maternal effect of the Y chromosome has been identified only in *Bombyx mori* (Tazima, 1954).

Preliminary chromosomal work on *E. s. westwoodi* revealed both intra- and inter-individual variability in chromosome number in male meiosis (Underwood *et al.*, in prep.). Of 113 cells from 13 individuals, the number of chromosomes varied from 52 to 94 (mean ± S.D. = 72.7 ± 8.9). Chromosome counts of different cells within the same individual varied from as few as four to as many as 22. Multivalents and lagging elements were present in many preparations, which suggests, at least in males, that meiosis is relatively unstable. We did not examine female meiosis. This chromosomal variability and the small size typical of lepidopteran chromosomes makes it difficult to determine directly whether sex is determined by a simple sex chromosome complement (XX = male, XO or XY = female) or by a complex of factors potentially involving sex-linked genes, autosomal genes and extragenomic elements. To date, *E. s. westwoodi* has failed to mate spontaneously or by hand-pairing in captivity. Until we are able to obtain matings in captivity, we will only be able to speculate about the mechanism producing this bias.

In diploid organisms with chromosomal sex determination, there are relatively few known mechanisms producing biased primary sex ratios. Cytoplasmic elements are the most common class of sex ratio distorters (e.g. Counce and Poulson, 1962; Johnson, 1977; Hurst, 1993). Transmission of these elements occurs through the female and, therefore, they are associated with female-biased sex ratios. Documented sex ratio biases in natural populations of other Lepidoptera are apparently of this type (Poulton, 1928; Chanter and Owen, 1972; Smith, 1975; Bowden, 1987). As the sex ratio bias in *E. s. westwoodi* is towards males, maternal cytoplasmic elements are probably not involved. Sex-linked meiotic drive genes are best described in *Drosophila* (e.g. James and Jaenike, 1990; Curtsinger, 1991; Carvalho and Klaczko, 1992) and two species of mosquito (Wood and Newton, 1991). In lemmings, an unusual sex-determining system results in some females being genetic males (XY) due to a gene located on some X chromosomes (designated X*), which suppresses the expression of the Y (male) (Fredga *et al.*, 1976; Bengtsson, 1977). However, for most cases of sex-ratio biases, the exact mechanism remains unknown.

As Lepidoptera females are heterogametic, the sex of the progeny is determined in female meiosis. As only one product of female meiosis forms an egg, any gene which ensures its placement in the oocyte rather than a polar body would be at a selective advantage. Although this idea is well-known (Charnov, 1982), no examples of this type of non-random segregation of sex chromosomes have been documented. It is assumed to be causal in cases of biased sex ratios in birds (e.g. Howe, 1977; Gowaty and Lennartz, 1985; Ligon and Ligon, 1990; but see Clutton-Brock, 1986), but it has not been demonstrated.

Non-random segregation of the sex chromosomes was presumably induced in two species of moth by elevating the air temperature during oviposition to 30–37°C (Seiler, 1920) and by delaying fertilization by 3–8 days (Golanski, 1968). Temperature as a causal factor in the bias described here does not seem likely under field conditions. In lepidopterans, female meiosis is arrested in metaphase I until after oviposition. As the female oviposits each egg, sperm are released from the spermatheca and one combines with the egg. The union of the gametes does not occur until after the exclusion of both polar bodies some time after oviposition. *Eucheira* oviposits its eggs on the undersurface of leaves. It is unlikely that there is sufficient variability in the microclimate associated with leaves in the field to explain the variability exhibited in sex ratio, although this has not been tested explicitly. We found no effect of delaying fertilization on sex ratio (Fig. 3).

By whatever mechanism sex is determined in *E. s. westwoodi*, the distribution of the sex ratio bias (Fig. 4) can shed some light on the nature of the sex-ratio distorter. As the bias is towards males, cytoplasmic elements are probably not involved. Cytoplasmic elements have generally been assumed to be only maternally transmitted; however, the recent discovery in a moth of a bacterium associated with spermatids and presumably transmitted via the male germ line (Wolf and Glatzel, 1996) is an interesting exception. This bacterium does not influence the sex ratio. A maternal cytoplasmic bacterium and a bacterial DNA sequence are involved in feminizing genetic males in the pill bug, *Armadillidium vulgare* (Crustacea: Isopoda) (Rigaud and Juchault, 1993). A masculinizing parasitic factor transmitted with the sperm in *E. s. westwoodi* could exist. However, in cases of maternal cytoplasmic sex-ratio distorters, the trend is for the production of unisexual broods via male embryo or gamete death, so that the population exhibits a bimodal distribution with modes near 50% male and 0% male. The primary sex ratio is clearly not bimodal in *E. s. westwoodi* and egg hatch failure is not great enough to account for the bias.

We are left with three potential mechanisms, two involving the X chromosome and one

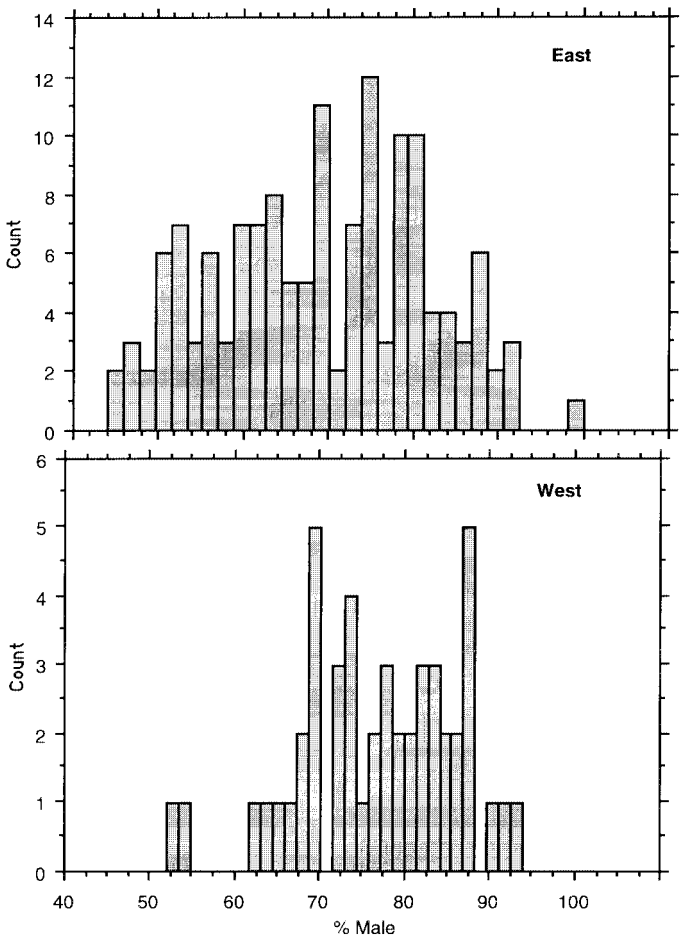

Fig. 4. Distributions of primary sex ratio for the eastern and western populations of *E. s. westwoodi*. Data for all years combined.

an autosome. If the sex-ratio distorter is located on the X chromosome as a masculinizing gene or as a sex-linked meiotic drive gene, it represents a selfish genetic element. In the absence of counterbalancing selection or suppressors and/or resistance genes, selfish genes will increase in a population until fixation. Sex-ratio distorters will cause the population to approach unisexuality and extinction. There has been no significant increase in primary sex ratio in either population of *E. s. westwoodi* during the period we have observed them (six generations). If the sex-ratio distorter is an autosomal masculinizing gene, it should be transmitted following normal mendelian rules and be subject to normal agents of selection. The non-binomial distribution and concomitant variability in sex ratio suggests a rather complex system involving the sex-ratio distorter, natural selection and suppressors and/or resistance genes.

In both populations, individuals have produced progeny that were not significantly male-biased. These individuals should be at a selective advantage unless producing a male-biased

progeny is adaptive. Over time, the population sex ratio should decrease. In 1990, the operational sex ratio was 65 and 76% male in the eastern and western populations, respectively; in 1997, eight generations later, the operational sex ratios were 71 and 73% respectively, suggesting a maintenance of the male-biased sex ratio. There is a rich literature on the ultimate cause of biased sex ratios; many, however, predict female-biased sex ratios, including local mate competition (LMC) (Hamilton, 1967), the multi-generation version of LMC, the haystack model (Bulmer and Taylor, 1980), and models proposed by Frank (1987) for social spiders. We discuss below the models that could explain the evolution and maintenance of a male-biased sex ratio.

Females should allocate equal resources to the production of males and females (Fisher, 1930). If the cost of males is less than the cost of females, allocating equal resources will lead to a male-biased progeny. In butterflies, the egg is already formed before sex is determined; therefore, unequal investment of resources to the sexes is implausible. Adult butterflies do not provide for their offspring, so differential parental care cannot be an evolutionary force in maintaining the biased sex ratio.

Local resource competition can lead to a male-biased sex ratio if males disperse more than their sisters and the progeny of sisters tend to compete with one another for resources (Clark, 1978; Silk, 1984). Indeed, *Eucheira* females in the eastern population do not disperse very far from the natal nest and individuals originating from nests in a particular tree often oviposit near one another, but in a tree different from the natal tree (Underwood, 1992). However, one prediction stemming from local resource competition is that, in populations of low density and little competition, the sex ratio would be less biased or not biased at all. For the past eight generations (1990–97), the western *Eucheira* population has always been at low densities, with only one or two nests per tree and relatively little defoliation, yet the operational sex ratio has been consistently more male-biased in the west than in the east. The eastern population commonly inhabits trees hosting in excess of 15 nests per tree and defoliation of many trees has occurred every year (Underwood, 1994a).

Kin selection could also maintain a biased sex ratio in organisms where interactions among relatives is sex-specific. Male offspring of the red-cockaded woodpecker help their parents in the rearing of broods; pairs without such helpers produce a male-biased progeny (Gowaty and Lennartz, 1985). In the green woodhoopoe, females are helpers and pairs lacking helpers produce female-biased broods (Ligon and Ligon, 1990). In African wild dogs, there is a sex ratio bias towards males and males act as helpers (Malcolm and Marten, 1982). There is reason to believe a functionally similar, but novel, phenomenon occurs in *Eucheria*. Recent laboratory experiments on sex-specific larval behaviour in *E. s. westwoodi* (Underwood and Shapiro, 1999) suggest that male larvae undertake most silk spinning for nest construction and depositing of foraging trails; this allows their sisters to conserve nitrogen and other nutrients that contribute to large egg masses. Male larvae are also the first to embark on foraging forays. Costs associated with searching for new foraging sites would be preferentially borne by the males, also leading to larger females. In a field experiment, male-biased nests produced heavier male and female pupae than female-biased nests. Hence, even if a nest comprises several sibships, males as well as females benefit from being in a male-biased group. Apparently, the sex-ratio distorter is maintained in these populations because male-biased groups are at a selective advantage compared to non-biased groups. However, extremely male-biased groups must suffer a reduction in fitness relative to moderately male-biased groups as more larvae die, and fewer males are successful in finding mates because there are not enough adult females in the population for all males to mate.

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REFERENCES

Bengtsson, B.O. 1977. Evolution of the sex ratio in the wood lemming, *Myopus schistiocolor*. In *Measuring Selection in Natural Populations* (F.B. Christiansen and T.M. Fenchel, eds), pp. 333–343. Berlin: Springer-Verlag.

Beutelspacher, C.R. 1983. Una nueva subspecie de *Eucheira socialis* Westwood (Lepidoptera: Pieridae). *An. Inst. Biol. Univ. Nal. Auton. de Mex. 54, Ser. Zool.*, **1**: 111–118.

Bowden, S.R. 1987. Sex ratio anomaly in an English Pierid butterfly. *Entomologist's Gazette*, **38**: 167–174.

Bulmer, M.G. and Taylor, P.D. 1980. Sex ratio under the haystack model. *J. Theor. Biol.*, **86**: 83–89.

Carvalho, A.B. and Klaczko, L.B. 1992. Age and sex-ratio expression in *Drosophila mediopunctata*. *Genetica*, **87**: 107–111.

Chanter, D.O. and Owen, D.F. 1972. The inheritance and population genetics of sex ratio in the butterfly *Acraea encedon*. *J. Zool.*, **66**: 363–383.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.

Clark, A.B. 1978. Sex ratio and local resource competition in a prosimian primate. *Science*, **201**: 163–165.

Clutton-Brock, T.H. 1986. Sex ratio variation in birds. *Ibis*, **128**: 317–329.

Counce, S.J. and Poulson, D.F. 1962. Developmental effects of the sex-ratio agent in embryos of *Drosophila willistoni*. *J. Exp. Zool.*, **151**: 17–31.

Curtsinger, J.W. 1991. X-chromosome segregation distortion in *Drosophila*. *Am. Nat.*, **137**: 344–348.

Díaz Batres, M. and Boudinot, J. 1986. *Eucheira socialis westwoodi* Beutelspacher, 1984: Contribution 'a sa biologie et a sa systématique (Lepidoptera: Pieridae). *Revue fr. Ent. (N.S.)*, **8**: 135–143.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Oxford University Press.

Frank, S.A. 1987. Demography and sex ratio in social spiders. *Evolution*, **41**: 1267–1281.

Fredga, K., Gropp, A., Winking, H. and Frank, F. 1976. Fertile XX- and XY-type females in the wood lemming (*Myopus schistiocolor*). *Nature*, **261**: 225–227.

Golanski, K. 1968. Effect of the age of fertilized eggs of *Bombyx mori* on sex determination. *Folia Biol.*, **16**: 191–198.

Gowaty, P.A. and Lennartz, M.R. 1985. Sex ratios of nestling and fledgling red-cockaded woodpeckers (*Picoides borealis*) favor males. *Am. Nat.*, **126**: 347–353.

Hamilton, W.D. 1967. Extraordinary sex ratios. *Science*, **156**: 477–488.

Hoffmann, C.C. 1910. Noticias de Humboldt acerca de los gusanos de seda indígenas de México con especial laborio de la 'Mariposa del Madroño' – *Eucheira socialis* Westw. In *Alexander von Humbolt*, pp. 134–158. Mexico: D.F. Muller Hnos.

Howe, H.F. 1977. Sex-ratio adjustment in the Common Grackle. *Science*, **198**: 744–746.

Hurst, L.D. 1993. The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. *Biol. Rev. Camb. Phil. Soc.*, **68**: 121–194.

Hurst, L.D., Atlan, A. and Bengtsson, B.O. 1996. Genetic conflicts. *Q. Rev. Biol.*, **71**: 317–364.

James, A.C. and Jaenike, J. 1990. 'Sex ratio' meiotic drive in *Drosophila testacea*. *Genetics*, **126**: 651–656.

Johnson, C. 1977. Evolution of sex ratios in the isopod, *Venezillo evergladensis*. *Evolution*, **31**: 603–610.

Karlin, S. and Lessard, S. 1986. *Theoretical Studies on Sex Ratio Evolution*. Princeton, NJ: Princeton University Press.

Kevan, P.G. and Bye, R.A. 1991. The natural history, sociobiology, and ethnobiology of *Eucheira socialis* Westwood (Lepidoptera: Pieridae), a unique and little-known butterfly. *The Entomologist*, **110**: 146–165.

Ligon, J.D. and Ligon, S.H. 1990. Female-biased sex ratio at hatching in the green woodhoopoe. *Auk*, **107**: 765–771.

Malcolm, J.R. and Marten, K. 1982. Natural selection and the communal rearing of pups in African wild dog (*Lycaon pictus*). *Behav. Ecol. Sociobiol.*, **10**: 1–13.

Nilsson, N.-O., Lofstedt, C. and Davring, L. 1988. Unusual sex chromosome inheritance in six species of small ermine moths (Yponomeuta, Yponomeutidae, Lepidoptera). *Hereditas*, **108**: 259–266.

Porter, A.H., Geiger, H., Underwood, D.L.A., Llorente-Bousquets, J. and Shapiro, A.M. 1997. Relatedness and population differentiation in a colonial butterfly, *Eucheira socialis* (Lepidoptera: Pieridae). *Ann. Ent. Soc. Am.*, **90**: 230–236.

Poulton, E.B. 1928. An all-female family of *Mylothris sprica*, Moschl., reared by Miss M.E. Fountaine from a company of larvae at Buea (about 3000 ft) in the Cameroons. *Proc. Roy. Ent. Soc.*, **2**: 75.

Rigaud, T. and Juchault, P. 1993. Conflict between feminizing sex ratio distorters and an autosomal masculinizing gene in the terrestrial isopod *Armadillidium vulgare* Latr. *Genetics*, **133**: 247–252.

Sachs, L. 1982. *Applied Statistics: A Handbook of Techniques*. New York: Springer-Verlag.

Scott, J.A. 1986. *The Butterflies of North America.* Stanford, CA: Stanford University Press.

Seiler, J. 1920. Geschlechtschromosenuntersuchungen an Psychiden. I. Experimentelle Beeinflussung der geschlechtsbestimmenden Reifeteilung bei *Talaeporia tubulosa* Retz. *Archiv für Zellforschung*, **15**: 249–268.

Silk, J.B. 1984. Local resource competition and the evolution of male-biased sex ratios. *J. Theor. Biol.*, **108**: 203–213.

Smith, D.A.S. 1975. All-female broods in the polymorphic butterfly *Danaus chrysippus* L. and their ecological significance. *Heredity*, **34**: 363–371.

Suomalainen, E. 1969. On the sex chromosome trivalent in some Lepidoptera females. *Chromosoma*, **28**: 298–308.

Tazima, Y. 1954. Mechanisms of sex determination in the silkworm, *Bombyx mori*. *Proc. Int. Con. Gen.*, **9**: 958–960.

Traut, W. and Marec, F. 1996. Sex chromatin in Lepidoptera. *Q. Rev. Biol.*, **71**: 239–256.

Underwood, D.L.A. 1992. Factors influencing oviposition behavior in the Mexican pierid butterfly, *Eucheira socialis*, on its host plant, *Arbutus xalapensis* (Ericaceae). Doctoral dissertation, University of California, Davis, CA.

Underwood, D.L.A. 1994a. Intraspecific variability in host plant quality and ovipositional preferences in *Eucheira socialis* (Lepidoptera: Pieridae). *Ecol. Ent.*, **19**: 245–256.

Underwood, D.L.A. 1994b. Methods for sexing Lepidoptera larvae using external morphology. *J. Lep. Soc.*, **48**: 258–263.

Underwood, D.L.A. and Shapiro, A.M. 1999. Evidence for division of labor in the social caterpillar *Eucheira socialis* (Pieridae: Lepidoptera). *Behav. Ecol. Sociobiol.*, **46**: 228–236.

Werren, J.H. 1987a. The coevolution of autosomal and cytoplasmic sex ratio factors. *J. Theor. Biol.*, **124**: 317–334.

Werren, J.H. 1987b. Labile sex ratios in wasps and bees. *BioSci.*, **37**: 498–506.

Wolf, K.W. and Glatzel, S. 1996. Intracytoplasmic bacteria in male germ cells of *Philudoria potatoria* L. (Lasiocampidae, Lepidoptera, Insecta). *J. Invert. Path.*, **67**: 279–288.

Wood, R.J. and Newton, M.E. 1991. Sex-ratio distortion caused by meiotic drive in mosquitos. *Am. Nat.*, **137**: 379–391.

Wrensch, D.L. and Ebberts, M.A. 1993. *Evolution and Diversity of Sex Ratio in Insects and Mites.* New York: Chapman & Hall.