Geographic Variation in Meiotic Instability in *Eucheira socialis* (Lepidoptera: Pieridae)

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Ann. Entomol. Soc. Am. 98(2): 227–235 (2005)

ABSTRACT We studied four populations of the pierid butterfly *Eucheira socialis* Westwood, located in the Mexican states of Sinaloa, Durango, Mexico, and Oaxaca. We found extreme geographic variation in spermatogenic abnormalities, including a variable number of chromosomes, irregular pairing of chromosomes, lagging chromosomes in metaphase I, lagging chromosomes in anaphase I, the production of micronuclei, and the failure of some spermatids to condense before maturation. We also present evidence suggesting the production of aneuploid sperm. These abnormalities correlate with previously reported male biases in the primary sex ratio of two of these populations. The population with the greatest bias in sex ratio also exhibited the highest frequencies of spermatogenic abnormalities. Because butterflies have female heterogamety and *Eucheira* females are XO, a possible mechanism linking the sex ratio biases and these abnormalities is nondisjunction of the X chromosomes during spermatogenesis, yielding disomic X sperm. We conclude that the most parsimonious explanation for the meiotic abnormalities in *E. socialis* is the presence of supernumerary chromosomes, which are associated with sex ratio biases in other systems.

KEY WORDS spermatogenesis, biased sex ratio, B chromosomes

THE ABILITY OF CELLS to divide in somatic tissues (mitosis) and in the formation of gametes (meiosis) is one of the most fundamental phenomena that unite most living organisms. The molecular basis for the faithful transmission of genetic material from one generation of cells to another or from one generation of organisms to the next involves numerous processes, including DNA replication, congression of condensed chromosomes on a metaphase plate, separation of chromatids by a spindle–kinetochore relationship, and cytokinesis to form daughter cells.

We now have a large literature describing both synthetically produced mutants and naturally occurring variants whose mitotic/meiotic cycles are different from normal cells. These include studies on plants (Aung and Walton 1987, Buckler et al. 1999), mice (Haaf et al. 1989, Agulnik et al. 1990), *Drosophila* (Ault and Lyttle 1988, Koehler et al. 1996, Basu et al. 1999), mosquitoes (Wood and Newton 1991), gypsy moth (Krider and Shields 1997), and lake trout (Disney and Wright 1990), to name a few. Meiosis is of particular interest because its products determine the raw material for the next generation.

We describe here geographic variation in a suite of meiotic abnormalities in four naturally occurring populations of the butterfly *Eucheira socialis* Westwood (Lepidoptera: Pieridae), including a variable number of chromosomes among cells within individuals and among individuals within populations, irregular pairing of chromosomes during metaphase I, lagging chromosomes in both metaphase and anaphase I, the production of micronuclei, and failure of sperm nuclei to condense before maturation. Two populations of *E. socialis* have significantly male-biased primary sex ratios (Underwood and Shapiro 1999) with a population located in the Mexican state of Sinaloa being significantly more male-biased than a population in the state of Durango. For the past 10 yr, mean primary sex ratios have been 70 and 80% male in the Durango and Sinaloa populations, respectively. Additionally, larval death is disproportionately male-biased even when taking the sex ratio bias into account (Underwood and Shapiro 1999). Butterflies have chromosomal sex determination with female heterogamety. If meiosis is normal, the sex ratio should be 1:1. Abnormalities in meiosis can result in altered sex ratios (reviewed in Wilkinson and Sanchez 2001) and death during development in a number of species (Counce and Poulson 1962, Agulnik et al. 1993, Hassold and Hunt 2001). We therefore hypothesized that chromosomal aberrations would occur more frequently in populations of *E.*

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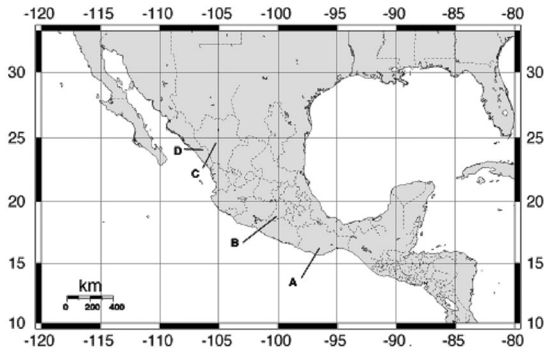

Fig. 1. Map of Mexico showing the locations of the populations studied. (A) Oaxaca. (B) Toluca. (C) Durango. (D) Sinaloa.

socialis that showed greater discrepancies in sex ratios and increased prevalence of larval death.

Materials and Methods

Collecting Sites and Dates. Fig. 1 shows the collecting sites for *E. socialis.* Larvae from Oaxaca were obtained from a population located on Highway 165 near San Jose del Pacifico in January 1999. We collected larvae from Toluca along Highway 134 near Temascaltepec in January 2001. Larvae from Durango were collected along Highway 40 between kilometers 64 and 95, and Sinaloa larvae were obtained from populations along the same highway at kilometer 213 in January 2002.

Control Species. We used the small white butterfly *Pieris rapae* L. (Pieridae) to test for the introduction of artifacts in our chromosome and sperm preparations. *P. rapae* is cytologically well described and exhibits normal spermatogenesis (Maeki 1958, Bigger 1976). We collected *P. rapae* from the campus at California State University, Long Beach, during August 2002. All protocols were applied to larvae of *P. rapae,* including digital analyses. Cells were scored in the same manner as was done for *E. socialis.*

Slide Preparation. For larvae from Durango, Sinaloa, and Toluca, we placed each larva in a folded card, which prevented lateral movement, and measured caterpillar body length to the nearest millimeter with a ruler. Larvae are largely quiescent during the day, and repeated measurements of test larvae found that larvae could be accurately measured in this way. We measured head capsule width to the nearest 0.1 mm by using an ocular micrometer. We dissected living sixth instar males to obtain their testes. Caterpillar sex can be determined by ventrally located sex-specific pits (Underwood 1994a). Exposed testes were immersed in a hypotonic treatment of tap water for $\approx$1 min. We then fixed the testes in two washes of 3:1 absolute ethanol and glacial acetic acid. We removed the outer testicular sheath and minced the remaining tissue into a cell suspension with fine forceps. Using a pipette, we then dropped 40 μl of suspension onto wet slides. The slides were air-dried and stained in 4% Giemsa for 20 min. After staining, the slides were rinsed under running tap water, allowed to dry, and made permanent with Permount (Fisher, Pittsburgh, PA) and a cover glass.

Cytometric Digital Analyses. We used a Nikon E800 microscope equipped with a Magna Fire charge-coupled device camera and for image analysis we used Image Pro Plus (Optronics Corp., Goleta, CA) digital analysis software. Lepidoptera spermatogenesis produces two types of sperm, nucleate (eupyrene) and anucleate (apyrene) sperm. We scanned slides for cells undergoing eupyrene metaphase I and anaphase I and for eupyrene sperm bundles with round nuclei. Not all classes of data could be obtained from each individual due to the sequential nature of spermatogenesis. The potential population-specific differences in spermatogenesis did not become clear until after Giemsa-stained slides had been made from the Oaxaca and Toluca populations. For this reason, we were unable to quantify DNA content by using standard Feulgen-DNA cytophotometry; instead, we used the two-dimensional area of these highly condensed chromosomes to estimate chromatin content. Metaphase cells in both the equatorial and polar views were analyzed. The equatorial view revealed the number of lagging chromosomal elements, whereas the polar view provided the total number and area of the chromosomal elements. For each animal, we counted the number of lagging elements seen in the first 20 anaphase and the first 20 equatorially aligned metaphase cells encountered. We analyzed images of eupyrene sperm bundles with round nuclei (telophase II) by counting the number of apparently normal spermatids, diffuse and/or fragmented spermatids, and micronuclei.

Statistics. To test for differences among populations for number of chromosomal elements per cell, area of chromosomal elements per cell, and number of spermatids and number of micronuclei per sperm bundle we used Friedman's analysis of variance (ANOVA) by ranks followed by Dunn's multiple comparison test. We calculated testes volume by using the formula of an ellipsoid, divided this value by larval length to control for differences in larval size, and used a one-way ANOVA to test for differences among populations. Variance in the number of lagging elements per 100 metaphase and anaphase cells could not be homogenized via transformation so these data were analyzed using a Kruskal–Wallis test with multiple comparisons (Zar 1996).

Results

Photographs of metaphase I cells from all four populations are shown in Fig. 2. Table 1 shows descriptive statistics and the results of two Friedman's tests by using chromosomal element number and total area from cells in metaphase I, polar view. We scored the *P. rapae* cells as we would have given no knowledge of the actual chromosome number. Whereas the reported number of chromosomes for *P. rapae* is 25, our methods found a mean of 24. Many cells contained a

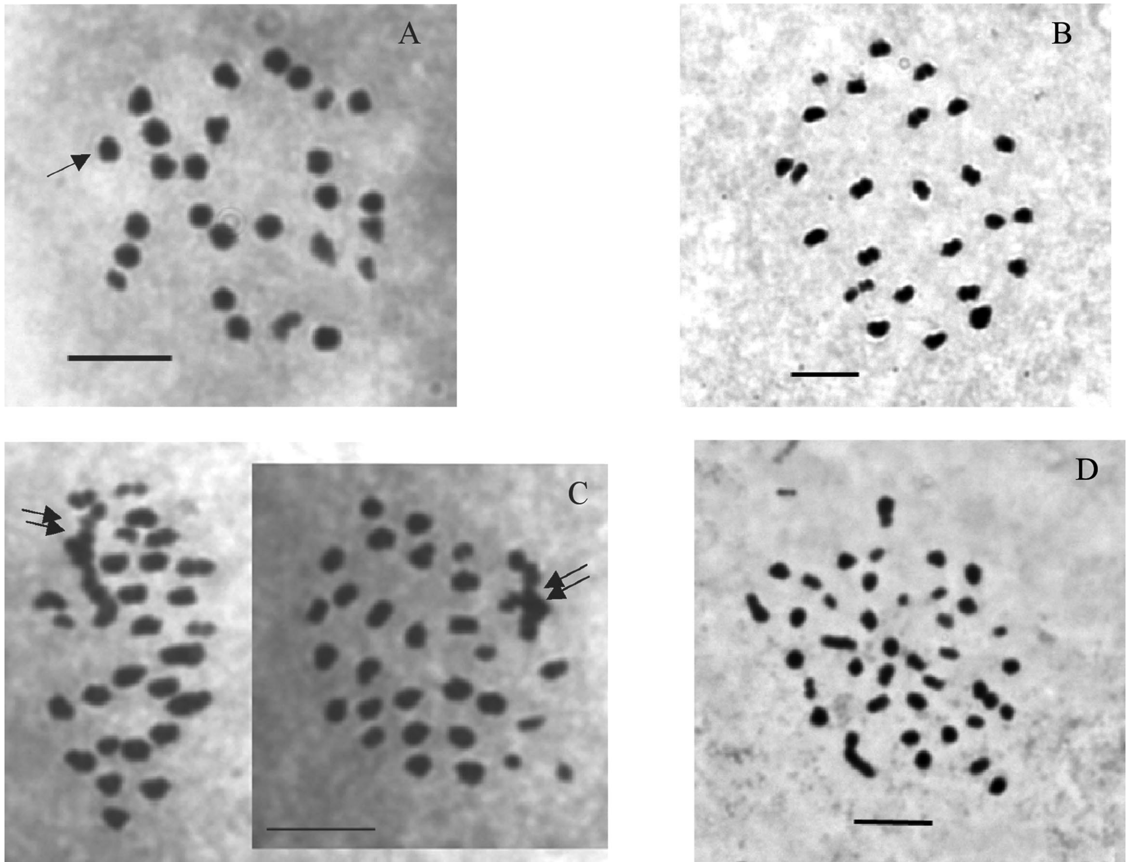

Fig. 2. Polar view cells in metaphase I. Larvae from Oaxaca (A) and Toluca (B) exhibiting normal chromosome associations; arrow in A indicates a typical normal bivalent. Larvae from Durango (C) and Sinaloa (D) showing abnormal chromosome associations. In C, double arrows indicate "exchange structures." Bars, 5 μm.

pair of chromosomes that were positioned close enough to one another to be scored as a single large element. However, even with this experimentally introduced error, *E. socialis* seems to consist of two, possible three, distinct populations based on chromosome number and element area. The low power of the nonparametric tests that we were forced to use did not allow us to statistically distinguish among these populations. Currently, the subspecies *E. s. socialis* includes the southern populations of Oaxaca and Toluca; these populations had fewer chromosomes than the northern populations of Durango and Sinaloa (chromosome number 27 versus $n = 33$ and $n = 39$, respectively). The two northern populations are presently in the subspecies *E. s. westwoodi* Beutelspacher, but our results suggest that their chromosome numbers are not the same (Table 1). Additionally, an earlier allozyme study found larvae from Durango, Sinaloa, and other southern populations to be as distinct from one another as many congeneric species are (Porter et al. 1997).

The total area of all of the elements in a metaphase I cell statistically split *E. socialis* into the northern and

Table 1. Number and total area of chromosome elements

Pop	No. animals scored	Mean no. of cells scored/animal	No. elements[a]		Total area of elements[b] (μm^2)	
			Mean (SE)	Range	Mean (SE)	Range
P. rapae	6	7.8	24.0 (0.2)a	20–25	33.3 (1.1)a	20.1–48.5
Oaxaca	5	9.6	27.0 (0.1)a,b	24–29	27.0 (0.1)a	21.3–40.5
Toluca	8	6.6	26.7 (0.1)a,b	25–31	30.1 (0.1)a	25.0–31.0
Durango	10	6.7	33.1 (0.6)a,b	26–43	41.5 (0.9)b	27.2–72.2
Sinaloa	13	5.5	39.1 (0.6)b	29–52	47.3 (1.7)b	28.3–98.7

Means with different letters are significantly different at the 0.05 level.
[a] Friedman test, $\chi^2 = 11.47$, $P = 0.0009$.
[b] Friedman test, $\chi^2 = 27.31$, $P < 0.0001$

Table 2. Number of lagging chromosomes per metaphase cell

Pop	No. animals scored	Mean no. cells scored/ animal	Mean (SE)[a]	Range	No. normal animals (%)
P. rapae	8	14.5	0 (0)a	0	8 (100)
Oaxaca	8	29.8	0.001 (0.001)a	0–0.01	7 (88)
Toluca	9	22.7	0.12 (0.04)a	0–0.34	4 (44)
Durango	18	39.6	0.62 (0.13)b	0–1.95	2 (11)
Sinaloa	13	19.9	1.69 (0.36)b	0–3.91	1 (8)

Means with different superscripts are significantly different at the 0.05 level.
[a] Kruskal–Wallis test, $\chi^2 = 35.7$, $P < 0.0001$.

Table 3. Number of lagging chromosomes per 100 anaphase cells

Pop	No. animals scored	Mean no. cells scored/ animal	Mean (SE)[a]	Range	No. of normal animals (%)
P. rapae	6	18.7	0 (0.0)a	0	6 (100)
Oaxaca	6	23.3	0.18 (0.13)a	0–0.83	3 (50)
Toluca	7	22.1	0.48 (0.18)a	0–1.31	1 (14)
Durango	15	25.3	0.54 (0.12)a	0–1.35	2 (13)
Sinaloa	13	18.7	1.47 (0.19)b	0.70–3.33	0 (0)

Means with different superscripts are significantly different at the 0.05 level.
[a] Kruskal–Wallis test, $\chi^2 = 27.2$, $P < 0.0001$.

southern subspecies (Table 1). The Sinaloa population not only had more elements, but the total area of these elements was greater than that of the Durango population, although the difference was not statistically significant.

The number of lagging chromosomes per metaphase cell was significantly greater in the Durango and Sinaloa populations than in *P. rapae* and the Oaxaca and Toluca populations (Table 2). Figure 3 shows examples of lagging chromosomes. All the *E. socialis* populations showed some lagging elements in some individuals, with Oaxaca having the highest percentage of individuals showing no laggards (seven of eight larvae were normal). As the mean number of lagging elements increased among the four populations, the number of individuals showing no laggards decreased. The range from larva to larva within a population also increased as the mean number of laggards increased.

The number of lagging elements per anaphase cell also was highly variable (Table 3). Although the means were between 0.18 and 0.54 laggards per cell for the Oaxaca, Toluca, and Durango populations, they were not significantly greater than the mean of zero found for *P. rapae* because of the extreme variability and the low power of the nonparametric test that was necessary to use. Sinaloa was the only population that was statistically distinguishable. All larvae scored from Sinaloa exhibited lagging elements, whereas the other *E. socialis* populations contained one or more larvae without laggards.

Sinaloa populations had significantly fewer spermatids per sperm bundle than *P. rapae,* but all other pairwise comparisons did not differ significantly (Table 4). Spermatids and micronuclei are shown in Fig. 4. *P. rapae* had a mean of 240 spermatids, suggesting that either our methods of preparing slides resulted in the loss of ≈16 spermatids or *P. rapae* spermatogenesis typically fails to produce a full set of 256 sperm. *P. rapae* Oaxaca and Toluca larvae did not differ in the number of diffuse spermatids per sperm bundle. The Durango and Sinaloa populations did not differ from each other, but they had a significantly greater number of diffuse spermatids than *P. rapae.* The number of micronuclei per sperm bundle was also significantly greater in the Durango and Sinaloa populations than in *P. rapae.* Although Oaxaca and Toluca populations both showed more micronuclei per bundle than *P. rapae,* these differences were not significant nor were the differences between the southern and northern populations of *Eucheira* statistically significant even though the mean differed by a factor of 10. Both southern populations contained individuals that did not produce micronuclei, whereas all individuals sampled from both northern populations had micronuclei.

P. rapae had the smallest sperm nuclei, whereas Sinaloa animals had the largest (Table 5); this is con-

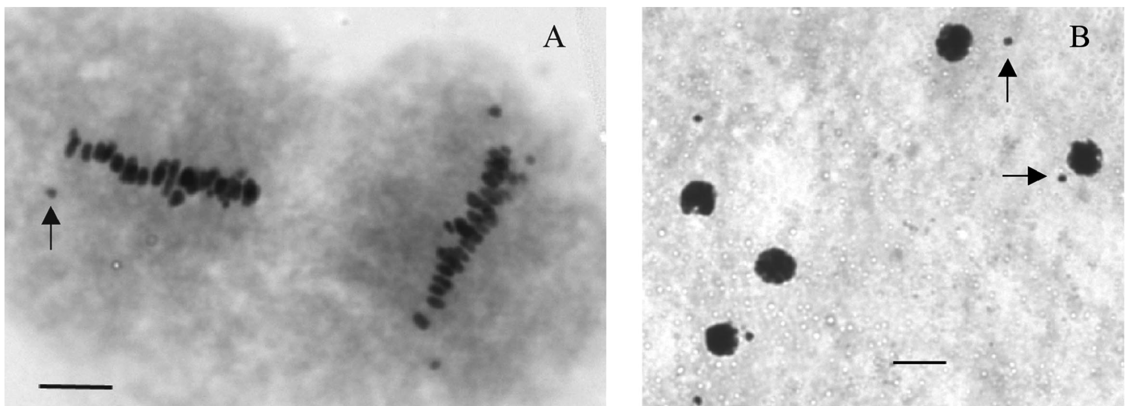

Fig. 3. Lagging chromosomes. (A) Equatorial view of cells in metaphase I laggards (Durango). (B) Larva from Durango showing lagging chromosomes in late anaphase. Arrows indicate laggards. Bars, 5 μm.

Table 4. Number of spermatids, diffuse spermatids, and micronuclei per sperm bundle

Pop	No. animals scored	Mean no. bundles scored/animal	No. spermatids[a]		No. diffuse spermatids[b]		No. micronuclei[c]	
			Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
P. rapae	7	4.0	240.1 (2.6)a,b	198–255	0.04 (0.0)a	0–1	0.6 (0.2)a	0–3
Oaxaca	6	9.2	238.0 (2.7)a,b	178–260	1.22 (0.2)a,b	0–9	1.9 (2.7)a,b	0–10
Toluca	8	6.5	242.4 (1.6)a	198–257	2.33 (3.3)a,b	0–8	12.5 (1.8)a,b	0–44
Durango	9	3.7	234.4 (2.9)a,b	185–256	8.21 (1.1)b	0–31	326.2 (18.2)b	143–582
Sinaloa	6	4.3	226.5 (4.3)b	163–256	9.85 (1.3)b	0–23	349.2 (34.7)b	133–1,032

Means with different superscripts are significantly different at the 0.05 level.
[a] Friedman test, $\chi^2 = 11.52$, $P = 0.02$.
[b] Friedman test, $\chi^2 = 19.36$, $P = 0.0007$.
[c] Friedman test, $\chi^2 = 19.04$, $P = 0.0008$.

sistent with our data on total area of chromosomes in polar-oriented metaphase cells with *P. rapae* having the smallest total area (Table 1). Spermatid size did not differ among the Oaxaca, Toluca, and Durango populations. The Sinaloa population had significantly larger spermatids than *P. rapae.* The Sinaloa population also showed the largest range with a few spermatids being twice as big as the smallest.

For nine larvae with data on both the number of laggards per anaphase I cell and the number of micronuclei, a simple regression revealed no significant relationship between these variables ($P = 0.07$). Sim-

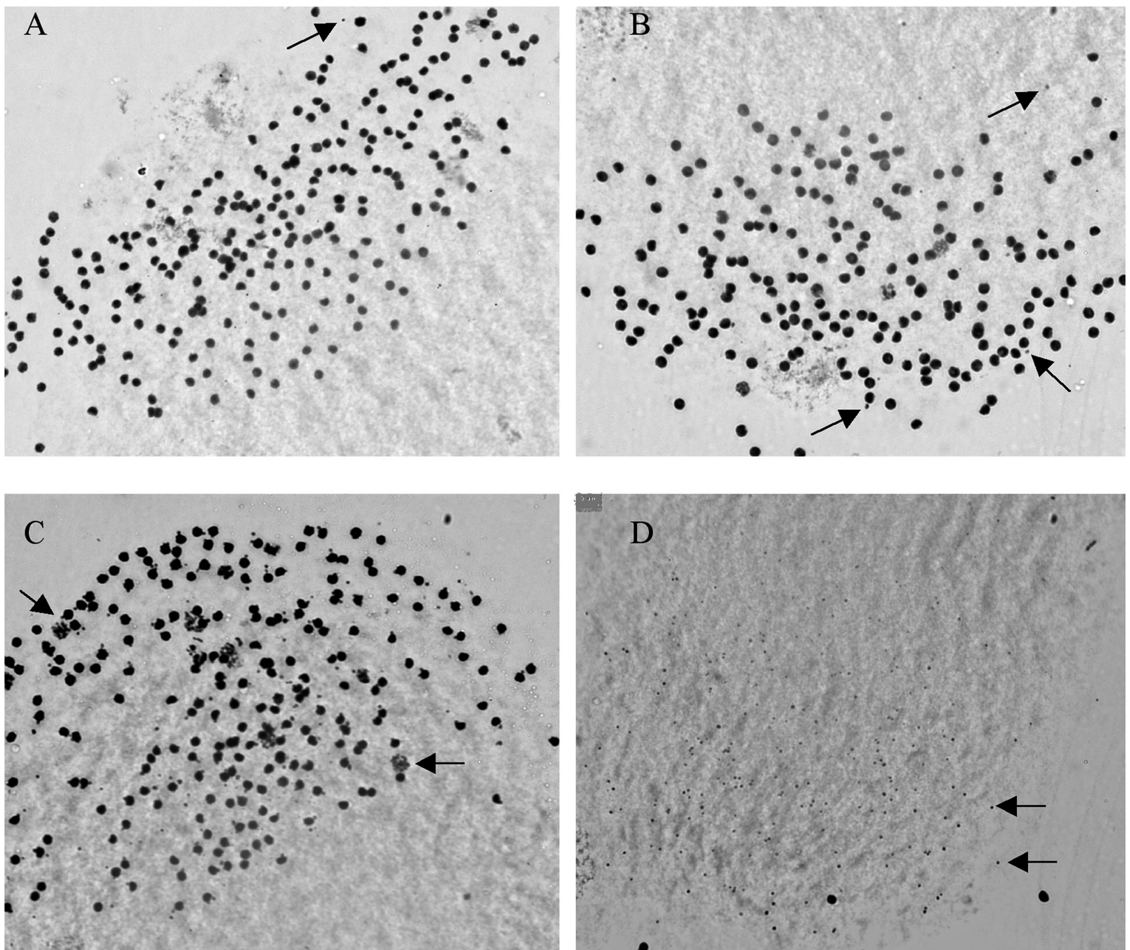

Fig. 4. Anterior end of sperm bundles showing spermatid nuclei (A) Larva from Oaxaca exhibiting few micronuclei and lacking diffuse spermatid nuclei. (B) Larva from Durango showing numerous micronuclei; two are indicated by arrows. (C) Larva from Sinaloa exhibiting hundreds of micronuclei and numerous diffuse spermatid nuclei (arrows). (D) Posterior end of a sperm bundle from a larva from Durango showing the shedding of micronuclei of various sizes; two are shown with arrows.

Table 5. Mean area of spermatid nuclei

Pop	No. animals scored	Mean no. bundles scored/animal	Mean area (SE)[a] (μm^2)	Range (μm^2)
P. rapae	3	3.0	2.8 (0.2)a	2.1–3.7
Oaxaca	3	3.0	3.5 (0.1)a,b	3.0–4.0
Toluca	3	3.0	4.1 (0.1)a,b	3.6–4.6
Durango	3	3.0	3.9 (0.1)a,b	3.4–4.5
Sinaloa	3	3.7	4.6 (0.3)b	3.5–7.0

Means with different superscripts are significantly different at the 0.05 level.

[a] Friedman test, $\chi^2 = 12.00$, $P = 0.0001$.

ilarly, the number of laggards per metaphase I cell and the number of micronuclei were not related ($n = 8$, $P = 0.55$). The number of laggards per metaphase and anaphase cell were significantly correlated ($n = 40$, $P = 0.001$); however, very little variation could be explained when one was regressed against the other ($r^2 = 0.23$). Laggards varied in size, but we did not control for this variation.

The morphological data are shown in Table 6. The length of the larvae did not differ among the three populations. However, head capsule width was significantly greater in the Durango population, followed by the Sinaloa population, and then the Toluca population. Tolucan larvae, although smaller, had proportionately larger testes when scaled by larval length. The Durango population was intermediate and the Sinaloa population showed the smallest testes. Many caterpillars from Sinaloa had testes so small it was not possible to get a sufficient amount of tissue with which to prepare slides.

Discussion

We report here considerable variation in abnormalities in male meiosis and spermiogenesis both within and among individuals and populations of the pierid butterfly *E. socialis.* These abnormalities included a variable number of chromosomal elements within populations and within individuals, irregular chromosome pairing during metaphase I, lagging chromosomes in metaphase and anaphase I, failure of some spermatid nuclei to condense, and the production of micronuclei associated with sperm nuclei. The frequency of all these abnormalities followed a similar pattern with larvae from Sinaloa being the most irregular followed by larvae from Durango, Toluca, and Oaxaca in decreasing order. Furthermore, not only were larvae from Sinaloa meiotically unstable but they also seemed to be developmentally retarded with respect to testes growth. We currently have no way of testing the possible effects of these abnormalities on male fertility because we have been unable to obtain matings in the laboratory.

Table 6. Larval length, head capsule width, and testes volume/larval length

Pop	No. animals scored	Mean larval length (SE)[a] (cm)	Mean head capsule width (SE)[b] (mm)	Mean testes vol/larval length (SE)[c]
Toluca	9	2.8 (0.08)a	3.0 (0.02)a	2.4 (0.17)a
Durango	70	3.0 (0.04)a	3.4 (0.03)b	1.7 (0.12)b
Sinaloa	15	3.0 (0.10)a	3.2 (0.04)c	1.0 (0.13)c

Means with different superscripts are significantly different at the 0.05 level.

[a] One-way ANOVA, $F = 1.7$, $P = 0.19$.

[b] One-way ANOVA, $F = 19.7$, $P < 0.0001$.

[c] One-way ANOVA, $F = 8.0$, $P = 0.0006$.

The primary sex ratio of the Sinaloa population is significantly more male-biased than the Durango population (Underwood and Shapiro 1999). In Sinaloa, 93–94% of the sibships sampled deviated significantly from sex ratio parity and the population mean was 75–79% male, whereas in Durango 55–69% of the sibships had sex ratios significantly different from 1:1 and the population mean varied from 68 to 71%. Unfortunately, we do not have data on the primary sex ratio from the populations in Toluca or Oaxaca. However, sex ratios of fourth instar groups and pupal sex ratios do not suggest that the primary sex ratio is biased in these populations (D.L.A. Underwood, unpublished data); these later stage sex ratios in Durango and Sinaloa are male-biased. Our findings here suggest that the mechanism producing the sex ratio bias is related to the degree of abnormality exhibited in male meiosis. In Lepidoptera, females are the heterogametic sex, so offspring sex should be determined by oogenesis. In most Lepidoptera, the Y chromosome is represented in female interphase cells as a heteropcynotic body resembling the Barr bodies seen in female mammals (Traut and Marec 1996). In 19% of 238 studied species of Lepidoptera, females lacked this sex-specific heterochromatin, indicating a XO/XX sex determination system (Traut and Marec 1996). We believe that *E. socialis* females are also XO because heterochromatin has never been seen in female interphase. If females are XO, then abnormalities in male meiosis that produced sperm that were disomic with respect to the sex chromosome would lead to a paternal origin of sex ratio bias. The results reported here support the idea that some sperm were aneuploid as metaphase I cells contained a variable number of elements and sperm nucleus area varied tremendously within bundles.

Intraspecific cytogenetic variation in chromosome number within the Lepidoptera is relatively common. In one survey, ≈12% of 880 lepidopteran species were reported as having a variable number of chromosomes (Robinson 1971). Five percent (45 species) were reported as varying by only one chromosome, 2% (20 species) varied by two chromosomes, 1% (12 species) varied by three, and 3% (26 species) varied by four or more. Much of this variation has been attributed to putative sibling species such as that seen in the *Philaethria dido* (L.) group (Suomalainen and Brown 1984) and variation due to the presence of B chromosomes (Bigger 1976, Pearse and Ehrlich 1979), which we discuss below. In those cases where chromosome number varied by three or more, fusion or fragmentation of chromosomes is cited as the possible mechanism (Robinson 1971, Emmel 1972, Emmel and Trew 1973). Lepidopteran chromosomes are holokinetic, which increases the probability that a chromo-

some fragment would possess some centromeric function and be captured by a spindle microtubule (Wolf 1996). Interestingly, we know of no study of these variable species that has examined all stages of spermatogenesis to document other possible abnormalities as reported here.

Recently formed interspecific hybrids in both plants (Comai 2000, Pikaard 2001) and vertebrates (Narain and Fredga 1997, Beçak and Beçak 1998, O'Neill et al. 1998) are characterized by univalents, trivalents, megachromosomes, and lagging chromosomes. Laboratory-produced interspecific hybrids within the Lepidoptera (Harrison and Doncaster 1914, Fontana 1976, Krider and Shields 1997) and the Orthoptera (Bidau 1991, Bidau and Marti 1998) also show these traits. However, we do not believe the populations of *E. socialis* that we studied are of hybrid origin for three primary reasons. First, we have not found any evidence for the existence of parental species from which our putative hybrids could come. *Eucheira* is a monotypic genus, quite removed evolutionarily from the remaining Pieridae. Second, dispersal of adults is extremely limited (Underwood 1992, 1994b), making it very improbable that individuals are flying from unknown populations into our study populations. Third, Dr. Richard Snow (personal communication) first documented the cytogenetic abnormalities in 1987 in Durango; persistence of these types of abnormalities for 16 generations would be exceedingly unusual considering how quickly these abnormalities disappear in other well studied interspecific hybrids.

The chromosomes involved in forming the exchange structures and perhaps some of the variability in bivalent number could be explained by errors occurring before metaphase I. Two possibilities are that multiple copies of the same chromosomes are produced via replication errors (Muzi Falconi et al. 1996, Kelly and Brown 2000) and/or nondisjunction events in mitosis within the embryonic germline immediately preceding meiosis as was documented in several species of grasshopper (Peters 1981, Talavera et al. 1990). Peters (1981) found that 20% of the male grasshoppers studied had 1–10 extra copies of one autosome; that these extra copies occurred as heteropcynotic bodies in interphase cells, lagged at metaphase I and anaphase I, and formed micronuclei; and their frequency responded to artificial selection in the laboratory. We have no data to suggest that either of these possibilities is present in *E. socialis,* although a polysomic X also could lead to a male-biased sex ratio. Furthermore, *Eucheira* sperm bundles that had 256 sperm nuclei still had many micronuclei implying that the DNA comprising the micronuclei was extra or that many of the sperm nuclei were aneuploid.

Perhaps the most parsimonious explanation for the meiotic instability seen in *E. socialis* is the presence of supernumerary chromosomes (B chromosomes). B chromosomes have been found in ≈2000 species of plants and animals and are defined as being dispensable and not necessary for the organism (Jones and Rees 1982, Camacho et al. 2000, Palestis et al. 2004). B chromosomes also are associated with the presence of heterochromatic bodies in interphase cells, nondisjunction, the formation of univalents and multivalents in metaphase I, laggards in anaphase I, and the production of micronuclei (Jones and Rees 1982, Bidau and Confalonieri 1988, Reed 1993, Clemente et al. 1994, Vicente et al. 1996). Although some B chromosomes seem to have originated from autosomes, some seem to be derived from sex chromosomes (reviewed in Camacho et al. 2000, Berend et al. 2001) as suggested by their tendency to associate with either the X or the Y chromosome during metaphase in some species. B chromosomes have been associated with both female-biased (Henderson 1988, Vicente et al. 1996) and male-biased (Nur et al. 1988, Beladjal et al. 2002) adult sex ratios. Although the mechanism leading to a sex ratio bias has not been determined, it is plausible that the presence of sex chromosome derived B chromosome(s) may interfere with normal disjunction of the sex chromosomes. Finally, in some animal systems, the presence of Bs has a negative fitness effect in the form of reduced production of normal sperm (Nur 1966, Remis and Vilardi 2004).

We found in *E. socialis* what seem to be B chromosomes, and cells in interphase commonly exhibited one or more heterochromatic bodies in both the Sinaloa and Durango populations, but not in the Toluca and Oaxaca populations. The highly condensed nature of butterfly chromosomes allows for only a gross distinction between large and small bivalents, hence our uncertainty as to the identity of putative B chromosomes. Additionally, the variability in size and number of chromosome elements and their irregular association in metaphase render conclusive identification problematic. If B chromosomes are present in *E. socialis,* this study shows a negative fitness effect in the form of retarded testes growth and sperm production in males of the populations that harbor Bs. Further studies using different techniques, for example, C-banding, which is often successful in identifying Bs, will be necessary to conclusively determine of potential role of B chromosomes in this system.

Acknowledgments

We thank the following students who assisted in slide preparation and data collection: Nathan Mudry, Steve Igic, Shariece Vallejo, and Chiharu Kumagai. We also thank Drs. E. Eldon, K. Young, and J.P.M. Camacho, who provided comments on earlier drafts of this article. We dedicate this article to the late S. Richard Snow, who first discovered these phenomena. We thank the National Geographic Society and the Office of Research at CSULB for providing funding, the USDA for providing a permit to import live animals, and México's Secretaría de Medio Ambiente y Recursos Naturales for a permit to conduct this study.

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Received 7 September 2004; accepted 11 December 2004.