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Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium

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Females of the butterfly *Acraea encedon* produce either entirely female offspring or males and females in an almost 1:1 sex ratio. The sex ratio produced is maternally inherited and was previously attributed to sex chromosome meiotic drive. We report that all-female lineages are associated with low egg-hatching rates and that the trait is cured by antibiotic treatment. We thus reject the hypothesis that this sex ratio bias is caused by a meiotically driven sex chromosome and, instead, propose that it is associated with a maternally inherited bacterium that kills males.

Keywords: *Acraea encedon*, male killing, meiotic drive, sex ratio.

Introduction

An allele is said to show meiotic drive when it 'cheats' during meiosis and is represented in more than 50% of gametes. The drive allele can spread through a population even if the cost to the individual of carrying this genomic parasite is considerable. If a drive allele is found on a sex chromosome, it will result in distortion of the sex ratio. Meiotic drive has been postulated to be an important force during a range of evolutionary changes, including speciation (Frank, 1991; Hurst & Pomiankowski, 1991), the evolution of crossing-over (Haig & Grafen, 1991) and the evolution of sex-determining mechanisms (Bull, 1979). For theories on the evolutionary importance of meiotic drive to have general applicability, it is first necessary to demonstrate that meiotic drive is a common phenomenon and that it occurs in a diverse range of taxa. To date, there is no definitive record of sex chromosome or autosomal meiotic drive in arthropods outside of the Diptera (see references in Hurst & Pomiankowski, 1991). The aim of this study was to investigate whether female-biased sex ratios recorded in the butterfly *Acraea encedon* (L.) are the result of sex chromosome meiotic drive, as has been proposed previously.

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Poulton (1914) first reported that some females of the African butterfly *Acraea encedon* produce only daughters, whereas others produce both sons and daughters. The sex ratio trait is maternally inherited (Owen, 1970). That this is a distortion of the sex ratio and not a change in sexuality to parthenogenesis is suggested by the finding that virgin females lay infertile eggs (Chanter & Owen, 1972). In butterflies, both the W chromosome (females are the heterogametic sex and carry ZW chromosomes) and the cytoplasmic genes (mitochondria and any intracellular endosymbionts) are maternally inherited and are thus selected to maximize the production and survival of female offspring. Chanter & Owen (1972) concluded that all-female broods were caused by a driving W chromosome distorting the primary sex ratio. However, Chanter & Owen's (1972) results distinguished neither which maternally inherited gene was causing the all-female broods nor whether all-female broods resulted from primary sex ratio distortion or from the death of male embryos or larvae. Male death could still result in all-female and normal sex ratio broods producing similar numbers of adults (as found by Chanter & Owen, 1972) if larval survival in all-female broods increases because of the benefits of cannibalizing dead brothers or through reduced competition in the absence of male siblings. The latter is likely, as survival is often strongly density-dependent in the laboratory.

Methods

In May 1997, adult *Acraea encedon* samples were collected from a single colony at Kagolomolo on the shores of Lake Victoria in Uganda, which was studied previously by Owen & Chanter (1969). The butterflies were taken to Watamu on the coast of Kenya, where the females laid eggs upon the host plant, *Commelina benghalensis*. Eggs and newly emerged larvae were counted to give egg hatch rates. Large clutches were photographed and counted at a later date. Up to five females were taken from each brood, mated and their egg hatch rates recorded. Up to three broods from each matriline were reared to adulthood, and the rest were discarded after hatching.

Adult females or larvae were treated with three antibiotics that had previously been shown to cure insects infected with cytoplasmic bacteria (Stouthamer *et al*., 1990). The concentrations used were the highest the butterflies would tolerate without refusing to feed. Fifth instar larvae were taken from some of the F_1 broods and placed on plants of *C. benghalensis* whose leaves had been dipped in a 5% (w/v) aqueous solution of tetracycline hydrochloride or a 2% (w/v) aqueous solution of sulphamethoxazole; a control group was fed on untreated plants. Caterpillars that refused to eat or pupated in under 2 days were discarded. Adult F_1 females were fed 10% (w/v) rifampicin in sugar solution daily for 6 days; a control group was fed on sugar solution alone.

Results

Among broods reared from wild females (n = 35), 86% produced only female offspring, whereas the remaining broods contained both males and females (Table 1). As seen from the sex ratio of the F_1 crosses, the trait was inherited by all the tested females, which is consistent with the maternal inheritance of sex ratio found by Chanter & Owen (1972). The parental generation of broods containing males (Table 1b) was significantly female biased (χ^2_1 = 10.13; $P<0.01$), but the sex ratio of the F_1 generation did not differ significantly from 1:1 (χ^2_1 = 0.80; NS). The cause of this sex ratio bias is not known.

The hatch rates of broods from all-female matrilines and normal sex ratio matrilines are significantly different (Mann–Whitney U-test: n_1 = 41, n_2 = 152; U = 1634; $P<0.0001$); this result is still significant if only broods reared to adulthood are included (Mann–Whitney U-test: n_1 = 11, n_2 = 43; U = 69.5; $P<0.0005$). The hatch rates of broods from

Table 1 Sex ratios produced by wild (parental) female *Acraea encedon* and in the subsequent (F_1) generation

	Parental females		F_1	
Matriline number	Male progeny	Female progeny	Male progeny	Female progeny
(a) All-female broods				
1	0	52	0	64
			0	97
2	0	62	0	80
3	0	70	0	20
4	0	48	0	81
5	0	31	0	67
6	0	52	0	104
10	0	15	0	58
11	0	25	0	80
15	0	5	0	49
16	0	8	0	19
17	0	3	0	3
26	0	5		
30	0	38	0	6
31	0	19	0	4
33	0	6		
35	0	35		
37	0	34	0	4
38	0	28		
39	0	17		
40	0	45	0	1
41	0	29		
42	0	41		
44	0	32		
45	0	9	0	3
49	0	11		
50	0	27		
55	0	11		
117	0	9	0	15
118	0	20		
119	0	8		
(b) Normal sex ratio broods				
7	4	12	5	3
			13	19
8	4	15	27	36
			27	38
			28	23
19	2	4	4	2
			15	8
34	12	22		
58	5	3		

Matrilines from which five or fewer individuals were reared over both generations are not shown. The eight largest normal broods (Table 1b) have heterogeneous sex ratios (χ^2_7 = 14.4; $P<0.05$).

all-female matrilines were never significantly greater than 50%, whereas broods from females in normal sex ratio matrilines had hatch rates of up to 100% (Fig. 1).

In all-female broods, there were large numbers of unhatched eggs visible when they had not been cannibalized. These eggs were black because of the tanned head capsule of a fully developed larval embryo. Black unhatched eggs were only rarely observed in normal sex ratio broods. Figure 1 also shows that many broods had very low hatch rates. Both normal and all-female broods with very low hatch rates were characterized by the presence of yellow unhatched eggs, presumed to be infertile. Yellow unhatched eggs were observed in similar proportions of all-female and normal broods (13 of 43 normal broods; 46 of 158 all-female broods; $\chi^2_1 = 0.02$; NS). Often, one region of a brood did not hatch and the eggs remained yellow; this contrasts with the even distribution of black unhatched eggs in all-female broods. Sib-matings did not have significantly lower hatch rates than outcrossed broods in normal matrilines (data not shown, Mann–Whitney *U*-test: $n_1 = 15$, $n_2 = 20$; $U = 131.5$; NS).

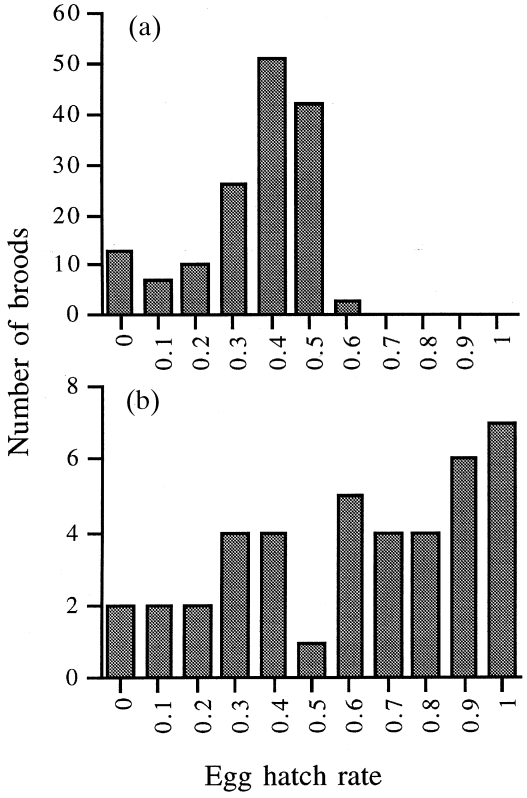

Fig. 1 Histogram of egg hatch rates from (a) all-female matrilines and (b) normal sex ratio matrilines of *Acraea encedon*. Brood sizes range from 22 to 470 eggs. Where one butterfly laid more than one clutch of eggs, they were combined as a single brood.

Table 2 Sex ratios produced by female *Acraea encedon* from all-female matrilines treated with antibiotics

Antibiotic	Matriline	Males	Females
Sulphamethoxazole	3	1	2
Sulphamethoxazole	3	7	6
Sulphamethoxazole	4	9	13
Sulphamethoxazole	5	12	15
Tetracycline	11	1	2
Rifampicin	1	0	38
Rifampicin	10	0	15
Rifampicin	5	0	5
Rifampicin	6	0	7

All the females are taken from the parental generation shown in Table 1. Untreated controls are the F_1 generation of the appropriate matriline shown in Table 1.

Table 2 shows the sex ratios produced by females from all-female matrilines that had been treated with antibiotics. Males were produced by females that had been treated with sulphamethoxazole or tetracycline as caterpillars. No successful treatments of normal sex ratio matrilines were completed to control for the endogenous effects of the antibiotic on sex ratio, although none is likely. The antibiotic rifampicin failed to cure the trait when fed to adults.

Discussion

Acraea encedon females produce either entirely female offspring or approximately equal numbers of males and females. This trait has now been found at high frequencies across Africa, from Sierra Leone to Tanzania, 6000 km to the east (Table 3). The sex ratio produced is maternally inherited, suggesting that all-female broods are caused by a selfish genetic element biasing sex allocation to enhance its own transmission. The hatch rate of broods from all-female matrilines is never significantly greater than 50%, whereas normal sex ratio lines have hatch rates of up to 100%. All-female broods in *A. encedon* are thus the result of the death of male embryos and are not caused by W chromosome meiotic drive distorting the primary sex ratio, as previously thought (Chanter & Owen, 1972). The fact that antibiotics cured all-female matrilines strongly suggests that the cause of all-female broods is a bacterium.

Sex chromosome meiotic drive has not been demonstrated conclusively in any arthropod taxa

Table 3 The frequency of all-female broods of *Acraea encedon* produced by wild females or wild-collected broods in the current and previous studies

Country	Proportion all-female	Broods reared	Reference
Tanzania	0.73	15	Owen & Smith (1991)
Sierra Leone	0.95	61	Chanter & Owen (1972)
Ghana	0.20	10	Chanter & Owen (1972)
Ghana	0.61	72	Gordon (1982)
Ghana	0.60	20	D. F. Owen & C. A. Clarke, unpublished data from 1982
Nigeria	0.70	26	Poulton (1914)
Uganda	0.77	13	Owen (1965); Chanter & Owen (1972)
Uganda	0.86	35	This study

outside of the Diptera (see list in Hurst & Pomiankowski, 1991). Sex chromosome meiotic drive was suspected to cause female-biased sex ratios in two species of butterfly, *Acraea encedon* and *Danaus chrysippus*, but the death of male embryos is now known to be the cause of sex ratio distortion in both these species (this paper and Owen & Smith, 1991).

The killing of males is, at worst, selectively neutral to a maternally inherited bacterium. The spread of male-killing bacteria is thought to result either from male-killing enhancing horizontal transmission of the parasite or through the death of males benefiting female siblings carrying clonal relatives of the bacterium (Hurst, 1991). Horizontal transmission has never been observed under laboratory conditions and, were this the selective advantage of male-killing, theory suggests that males would be more likely to be killed during late larval instars (Hurst, 1991).

Male death may benefit female siblings by preventing inbreeding. Owen *et al.* (1973) calculated from the population sex ratio that, in most female-biased populations encountered, the majority of females must remain unmated. This was confirmed in the field, where most wild females were found to be unmated, and unmated females were observed laying infertile eggs. It is unlikely under these conditions that inbreeding would be disadvantageous; inbreeding may benefit uninfected females by increasing their chances of mating. It should be noted that Owen *et al.*'s (1973) work confused the two species *A. encedon* and *A. encedana* Pierre, so it is not possible to tell to which species their results apply (Owen & Smith, 1991).

The final hypothesized advantage of male-killing is that resources are reallocated from the dead males to their sisters, either through sibling cannibalism or through a reduction in competition. This possibility finds support in Chanter & Owen's (1972) observation that females in all-female matrilines developed significantly faster than females in normal matrilines. Chanter & Owen (1972) also found that the numbers of adults in all-female and normal broods were not significantly different, which suggests that infected females have higher egg-to-adult survival. An alternative interpretation of the greater survival and growth rates of infected females is that the bacterium provides some metabolic benefit to females. Parasitic and mutualistic roles need not be mutually exclusive in endosymbionts.

In contrast to cytoplasmic genes that distort the primary sex ratio, early male-killing is found to be associated with a taxonomically diverse range of both arthropod hosts and bacterial parasites (Hurst *et al*., 1997). Hurst *et al.* (1997) hypothesized that the critical factor favouring the evolution of male-killing behaviour is a host ecology that makes antagonistic interactions between siblings or sib cannibalism likely. The finding of male-killing in *A. encedon* lends further support to the importance of host ecology in the evolution of male-killing. *Acraea encedon* typically lays clutches of between 50 and 300 eggs, and newly emerged larvae often cannibalize unhatched eggs. The larvae gradually disperse into smaller groups and only become solitary in the final instar. This ecology clearly provides opportunities for both egg cannibalism and sibling competition that may favour the spread of male-killers.

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