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Cover picture from the paper by Davies et al.
Hybridizing butterflies: *A amathea* x *A fatima*.
(Photo: R. E. Silberglied and A. Aiello.)

Speciation in two neotropical butterflies: extending Haldane's rule

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SUMMARY

Anartia fatima and *A. amathea* form a hybrid zone in Panamá where F1 and back-cross hybrids are found. Crosses were carried out to determine the nature of any reproductive isolation between these two butterflies. A novel analysis demonstrated both strong assortative mating among the pure forms and an unusual example of Haldane's rule: F1 hybrid females (the heterogametic sex) from the cross *A. amathea* (female) × *A. fatima* (male) have a reduced tendency to mate. Historically, Haldane's rule has been restricted to hybrid mortality or sterility and most studies have concentrated on taxa (predominantly *Drosophila*) between which strong barriers to gene flow already exist. Our data suggest that Haldane's rule might be extended to cover any decrease in hybrid fitness and that mating propensity may provide a sensitive and comparable means of assessing such decreases. Other barriers to gene flow were also evident in *Anartia*: F1 hybrid females have reduced fertility (also a Haldane effect) and larval survivorship was greatly reduced in F2 hybrids of both sexes. These examples of hybrid disruption are expected under the dominance theory of Haldane's rule but do not exclude other explanations.

1. INTRODUCTION

Speciation studies are dominated by a single genus, *Drosophila*. In their review of hybridization data, Wu & Davis (1993) listed 225 experiments in *Drosophila* but only 53 in birds and 55 in Lepidoptera. The bias is much stronger when one takes into account the detail in which hybrid incompatibilities have been studied. Although there are good reasons for choosing *Drosophila*, such taxonomic specialization is limiting. For example, Haldane's rule is the only robust generality in speciation: it states that when one sex of hybrid is absent, rare, or sterile, it is usually the heterogametic sex (Haldane 1922). Haldane's rule would remain undiscovered without data from taxa where the heterogametic sex is female (e.g. birds and Lepidoptera) as well as other heterogametic male taxa (e.g. mammals). More data from butterfly hybridizations are desirable, partly because Lepidoptera have heterogametic females, but also because they have small X-chromosomes. (N.B. For simplicity, we use 'X' and 'Y', rather than 'Z' and 'W', to describe Lepidopteran sex chromosomes.) The X-chromosome constitutes approximately 25% of the *Drosophila* genome (Ashburner 1989) but only some 5% of a typical Lepidopteran genome (Robinson 1971). This is significant because X-linked genes seem disproportionately important in Haldane's rule (Coyne & Orr 1989*b*).

Haldane's rule evolves early in the divergence of *Drosophila* species (Coyne & Orr 1989*a*) and possibly in Lepidoptera also, since Haldane effects are often found among closely related butterflies (Oliver 1979; Grula & Taylor 1980*b*; Hagen & Scriber 1995). 'Dominance theory' is the most likely explanation for Haldane's rule and the large-X effect (Davies & Pomiankowski 1995; Turelli & Orr 1995). Dominance theory assumes nothing special about X-linked genes *per se*, the importance of the X-chromosome is its hemizygosity (Turelli & Orr 1995). According to dominance theory, most genes contributing to hybrid disruption are recessive and are therefore expressed only in F1 hybrids when X-linked and in the heterogametic sex, hence Haldane's rule. In F2 hybrids, incompatibilities due to homozygous autosomal recessives can be expressed in either sex, hence 'hybrid breakdown'(Dobzhansky 1937). In Lepidoptera F1 hybrids, Haldane's rule should be relatively unimportant as the X-chromosome represents a small proportion of the genome. F2 hybrid breakdown should often evolve first and affect both sexes. In spite of this prediction, Haldane's rule is

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common in Lepidoptera. More rapid evolution of X-linked recessive genes could also explain Haldane's rule (Charlesworth *et al.* 1987). A 'special-X' theory of speciation may be necessary for Lepidoptera as many of the characters that differentiate species are X-linked (Sperling 1994).

Here we present the results of a hybridization programme between two butterflies, Central American *Anartia fatima* and South American *A. amathea* (Lepidoptera: Nymphalidae), which meet at a narrow hybrid zone, about 10 km wide, in eastern Panamá. Although *A. fatima* and *A. amathea* are considered to be separate species, reproductive isolation is incomplete: F1 and back-cross hybrids (identified through wing patterns) are found in the area of overlap. Pure forms, however, predominate and despite their similar ecology, the species have evolved morphological (Silberglied *et al.* 1979) and genetic differences. *Anartia fatima* and *A. amathea* are sister species whose genetic distance (Nei 1978), determined through allozyme electrophoresis, is $D_N = 0.20$ (Stuart Hackwell, N.D. & J.M., unpublished data). The genetic distance to their next closest relative is $D_N = 0.47$ (*A. amathea* to *A. chrysopelea*). Our study describes the barriers to gene flow that separate these incipient species and assesses the importance of Haldane's rule incompatibilities.

2. MATERIALS AND METHODS

(a) *'Premating' isolation*

Mate choice was investigated in six multiple mate choice experiments performed in 22.5 m^3 outdoor cages on Barro Colorado Island, Panamá (by R.E.S. & A.A.). We use the abbreviations: *A* for *A. amathea*, *F* for *A. fatima*, *AF* for F1 hybrid offspring of female *A. amathea* × male *A. fatima*, and *FA* for F1 hybrid offspring of female *A. fatima* × male *A. amathea*. Two experiments involved the pure species reared either from sympatric (hybrid zone near Santa Fé, Darién) or allopatric (*A. amathea* from Bahía Solano, Colombia; *A. fatima* from Panamá Canal area) stocks. In the other four experiments, pure species were tested against each type of reciprocal F1 hybrid (reared from allopatric stock). Equal numbers of each sex and of each pair of species or hybrid type were caged together. In the absence of choice or differences in mating propensity, the four possible crosses should occur with equal frequency. Each experiment was carried out over several days using sexually active males and virgin females ($\geqslant$ 3 days' old). The number of butterflies in the cage varied among and during experiments (between 32 and 100), but experiments were kept balanced both for sex and type. Mated females and dead individuals were replaced (males will mate repeatedly, females will not). Experiments were monitored constantly from 10:00 to 15:00 hrs (when *Anartia* are most active) and every 10 min during other daylight hours (*Anartia* are inactive at night).

The results are shown as 2 × 2 tables of mating frequency among species/types (figure 1). Typically, the analysis of such data is beset by two problems. First, mate choice and mating propensity of the two sexes are confounded. Second, information is wasted by analysing each experiment separately (components of mating behaviour should be similar in different experiments that involve the same strains). We overcome these difficulties by using a likelihood analysis to compare different models of mating. For each 2 × 2 table, mating behaviour can be viewed as consisting of three parameters: mating propensity of females (V) measured as a relative probability of mating between the two types of females, a similar mating propensity of males (W), and an additive interaction term which measures mate choice (I). Under this model, for two (hypothetical) populations 'i' and 'j', the probability of i × i matings is: $p_{ii} = [V_i/(V_i+V_j)][W_i/(W_i+W_j)]+I_{i\times j}$; similarly $p_{ij} = [V_i/(V_i+V_j)][W_j/(W_i+W_j)]-I_{i\times j}$, and so on. All V, $W = 1$ under a null hypothesis of equal mating propensity; similarly, random mating would have all $I = 0$.

Twice the difference in log likelihood ($\Delta\log L$) between two models asymptotically follows a χ^2 distribution. Support limits, where the likelihood drops to two units below the maximum, are asymptotically equivalent to 95% confidence limits and can be used to assess the reliability of a parameter estimate (Edwards 1972). Each 2 × 2 experiment has three parameters, making a total of 18 parameters over the six experiments. Because 18 is the maximum possible number of parameters, mating interactions between i females and j males cannot be separated from those between j females and i males, nor can male and female choice be separated using these data. Separate experiments or other behavioural data would be required to distinguish between male and female choice, or between reciprocal mating interactions.

Sympatric and allopatric $A \times F$ tests did not differ significantly in parameter values ($\Delta\log L = 3.5$, 3 d.f.) so the parameters from these two tests were combined giving 15 d.f. Non-significant decreases in likelihood were also obtained assuming that mating propensities for each strain and sex (V, W) did not differ between experiments ($\Delta\log L = 2.7$, 4 d.f.) and that mate choice was equal in all experiments involving parentals and hybrids (i.e. pure × hybrid parameters $I_{A\times AF} = I_{A\times FA} = I_{F\times AF} = I_{F\times FA}$, $\Delta\log L = 1.3$, 3 d.f.); after combining these parameters, we obtain the 'best model' with eight parameters. Expected values for this model are shown in figure 1. Maximum likelihood parameter values and support limits were found manually, using a computer program to calculate likelihoods. To check for additional peaks in the likelihood surface, 8.32×10^8 parameter values were iteratively checked in the final model across the range of likely values. No higher peaks were found.

(b) *'Postmating' isolation*

Mated females were placed in small cages with the larval food plant, *Blechum brownei* Juss. (Acanthaceae), which is distributed throughout the neotropics and is used by both *A. fatima* and *A. amathea* (Silberglied *et al.* 1979). Eggs were placed in separate Petri dishes in lots of twenty. Each day the dishes were cleaned and the larvae were fed with fresh *B. brownei* leaves. Upon reaching the third stadium, larvae were transferred to small cages. All broods received similar treatment and were reared outdoors. The division into separate lots makes it unlikely that any one brood was disproportionately affected by a viral infection or other external factors.

For each brood we recorded the numbers of eggs, larvae, and male and female adults (Appendix 1), and calculated the proportion of eggs hatched (egg hatch), the proportion of larvae surviving to eclosion (larval survivorship), and the sex ratio at eclosion. The influence of cross type on mean egg hatch (arcsine transformed) and mean larval survivorship was tested by one-way ANOVA. As F1 hybrids have pure parents, any reduction in egg hatch results from genomic incompatibilities in the hybrid. In back-crosses, however, reduced egg hatch could also be caused by the sterility of the parents. To test for sex differences in sterility, egg hatch of back-crosses involving a hybrid dam (BC-1) were compared with those of back-crosses involving a hybrid sire (BC-2). These two classes were also compared for larval survivorship.

(*a*) sympatric cross

	A male	*F* male
A female	92 (93.3)	16 (20.1)
F female	40 (33.6)	64 (65.0)

(*b*) allopatric cross

	A male	*F* male
A female	44 (45.8)	14 (9.8)
F female	10 (16.5)	36 (31.9)

(*c*) *amathea* × (*amathea* × *fatima*)

	A male	*AF* male
A female	22 (19.5)	25 (26.2)
AF female	3 (2.3)	3 (5.0)

(*d*) *fatima* × (*amathea* × *fatima*)

	F male	*AF* male
F female	2 (4.5)	10 (9.0)
AF female	0 (0.6)	4 (1.9)

(*e*) *amathea* × (*fatima* × *amathea*)

	A male	*FA* male
A female	5 (3.8)	3 (3.2)
FA female	2 (3.4)	4 (3.6)

(*f*) *fatima* × (*fatima* × *amathea*)

	F male	*FA* male
F female	5 (5.8)	7 (7.2)
FA female	7 (5.9)	9 (9.1)

Figure 1. Mate choice experiments. Results for six 2 × 2 multiple mate choice experiments are shown. Expected values based on the 'best' model (figure 2) of mating behaviour are shown in parentheses. *AF* = *A. amathea* female × *A. fatima* male, *FA* = *A. fatima* female × *A. amathea* male.

Larval survivorship was compared in two further classifications of back-cross hybrids, according to (i) the specific origin of their Y-chromosome and cytoplasm (we had no marker for the X-chromosome), and (ii) which species was the pure parent (F1 hybrids back-crossed to *A. amathea* will produce hybrids that are 75% *A. amathea* and 25% *A. fatima*, and vice versa for F1 hybrids back-crossed to *A. fatima*).

3. RESULTS

(*a*) *Mate choice*

Assortative mating did not differ between sympatric and allopatric populations ($\Delta \log L = 3.5$, 3 d.f.; figure 1*a*, *b*), therefore assortative mating parameters between pure species were pooled. Intraspecific matings were about three times as frequent as interspecific matings, although neither species distinguished hybrids from their own type (figure 2*a*). It is impossible on the basis of mating data alone to determine whether male or female choice was responsible for the assortative mating (see Materials and Methods). However, males regularly courted females of both species and even attempted to copulate with heterospecific males, suggesting a stronger role for female choice. In a separate study, however, male *A. amathea* were reluctant to court *A. amathea* females with red bands painted black, yet *A. amathea* females mated readily with black-painted *A. amathea* males (Silberglied 1984), suggesting that male choice could also be important.

(*b*) *Mating propensity*

Analysis of F1 hybrid mating propensity provided an unusual example of Haldane's rule: hybrid females from the cross *A. amathea* female × *A. fatima* male had strongly reduced mating propensity compared with other females–one-sixth of that of a typical *A. amathea* female (figure 1*c*, *d*; figure 2*b*). We have no quantitative behavioural data on the vigour of hybrid females other than their mating frequency, although the low mating propensity of *AF* females seemed to correlate with a general inactivity and tendency not to fly. Females of the reciprocal cross mated with normal frequency and appeared to have normal activity. The only other significant difference in mating propensity was that *A. fatima* males mated about one-third less often than *A. amathea* males.

(*c*) *Egg hatch*

One-way ANOVA revealed a significant effect ($F = 9.97$, $p < 0.0001$) of cross type on mean egg hatch among parental, F1 hybrids, back-crosses with a hybrid dam (BC-1), back-crosses with a hybrid sire (BC-2) and F2 hybrids (figure 3). Tukey–Kramer-corrected *post hoc* tests ($p < 0.05$) showed that the significant effect was due to the decreased egg hatch of BC-1 and F2 broods compared with parental, F1 and BC-2 broods. The lower mean egg hatch of back-crosses with

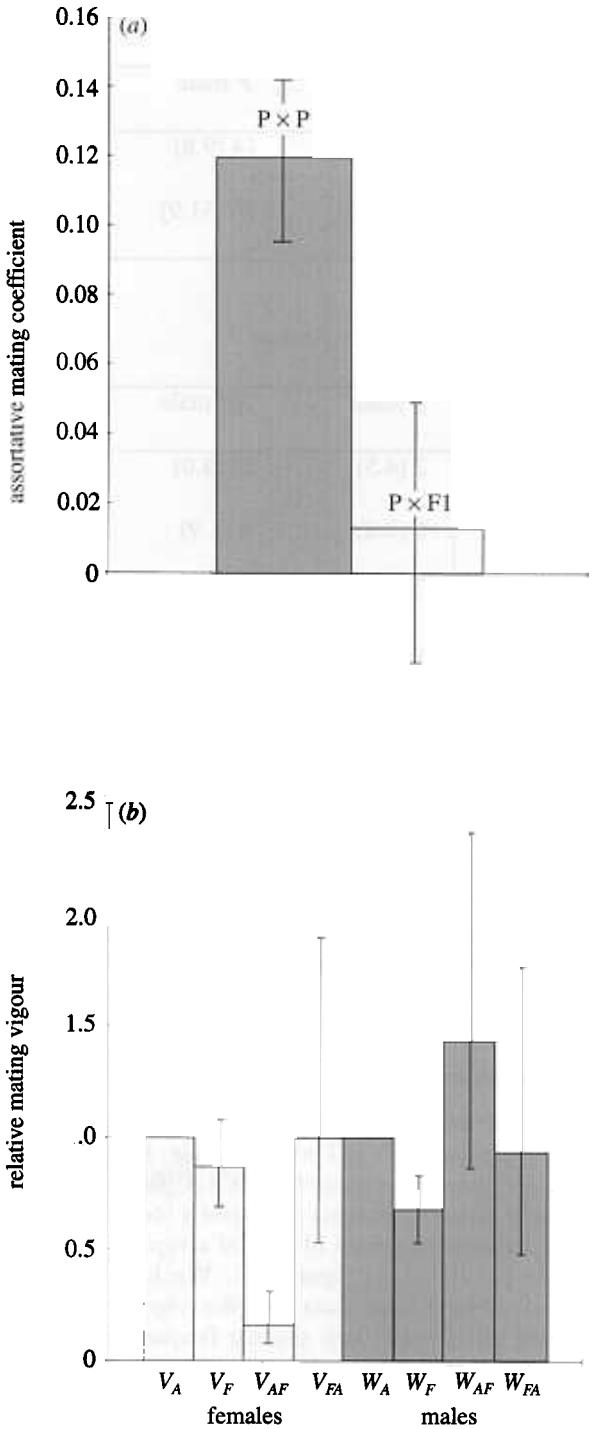

Figure 2. Mating behaviour. Each estimated mating parameter is shown with its support limits—asymptotically equivalent to 95% confidence limits. (*a*) Strong assortative mating ($I \gg 0$) was found in parental (P × P) tests (figure 1*a*, *b*), but there was no evidence of mate choice in parental × hybrid (P × F1) tests (figure 1*c*–*f*). (*b*) Mating propensity is shown relative to *A. amathea* females ($V_A = 1$) and males ($W_A = 1$). Mating propensity was greatly reduced in *AF* females ($V_{AF} = 0.16$) and somewhat lower in *F* males ($W_F = 0.67$). No other mating propensities differed significantly from those of *A. amathea*. Maximum likelihood estimates of mating parameters, support limits, and tests of null hypotheses V, $W = 1$ and $I = 0$ are given in table 1.

a hybrid dam (BC-1) compared to those with a hybrid sire (BC-2) clearly did not depend upon the direction of the cross. Mean egg hatch was 0.24 in the two broods

Table 1. *Maximum likelihood estimates of mating parameters, support limits, and tests of null hypotheses* V, $W = 1$ *and* $I = 0$

(Note: $I_{P/P} = I_{A/F}$; $I_{P/H} = I_{A/AF} = I_{A/FA} = I_{F/AF} = I_{F/FA}$; all tests with 1 d.f.; probability in significance tests based on χ^2 approximation as follows: n.s., $p > 0.05$, * = $p < 0.05$.)

parameter	value	support limits	$\Delta \log L$	p
	0.87			n.s.
	0.16			< 0.001
	1.00			n.s.
	0.67			< 0.01
	1.43			n.s.
	0.93			n.s.
	0.120			< 0.001
	0.013			n.s.

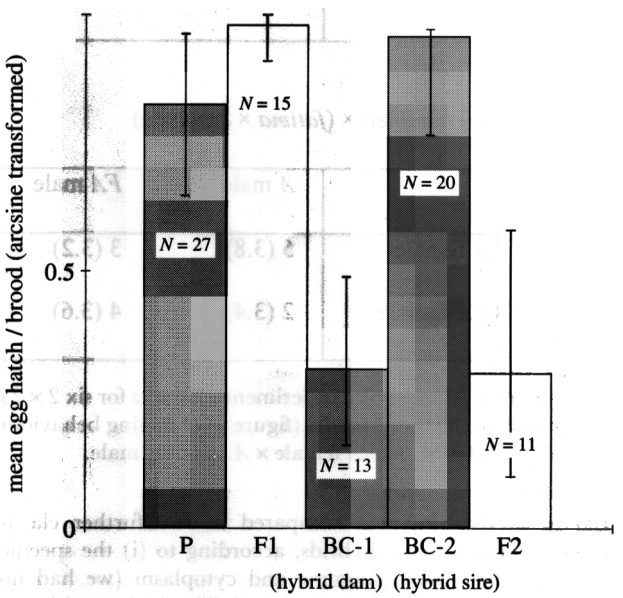

Figure 3. Egg hatch in crosses. Mean egg hatch and 95% confidence limits for parental (P), F1 hybrid (F1), F1 hybrid dam back-cross (F1 × P = BC-1), F1 hybrid sire back-cross (P × F1 = BC-2) and F2 hybrid (F1 × F1 = F2).

with an *AF* hybrid dam (in which matings and subsequent eggs are difficult to obtain because of inactivity) and 0.33 in the 11 broods with *FA* hybrid dams. By comparison, mean egg hatch was 0.65 in parental broods and 0.75 in broods with a hybrid sire (BC-2). The most likely explanation for the lower egg hatch of BC-1 and F2 broods is that hybrid dams have a lower fertility than pure dams, i.e. hybrid females are partially sterile.

(*d*) *Larval survivorship*

One-way ANOVA also revealed a significant effect ($F = 4.81$, $p < 0.01$) of cross type on mean larval survivorship among parental, F1 hybrids, BC-1, BC-2 and F2 hybrids (figure 4). Tukey–Kramer-corrected *post hoc* tests ($p < 0.05$) showed that the significant effect was largely due to F2 hybrid breakdown: F2 hybrids were markedly inferior to parental and F1 hybrids. Five of the F2 broods resulted from sibling matings but there was no evidence of inbreeding

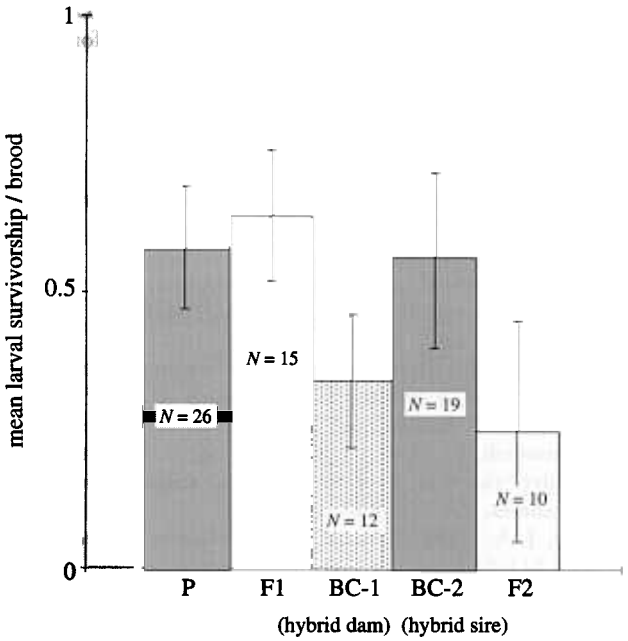

Figure 4. Larval survivorship in crosses. Mean larval survivorship between egg hatch and eclosion is shown for parental (P), F1 hybrid (F1), F1 hybrid dam back-cross (F1 × P = BC-1), F1 hybrid sire back-cross (P × F1 = BC-2) and F2 hybrid (F1 × F1 = F2). Error bars represent 95% confidence limits.

depression as broods from sib-matings had higher larval survivorship (0.19) than outbred broods (0.14). Broods with a hybrid dam (BC-1) had lower mean larval survivorship than broods with a hybrid sire (BC-2). These differences were evident from comparisons with the survivorship of F1 and F2 broods (figure 4). Classing back-crosses according to whether they had a hybrid dam or a hybrid sire does not identify any particular hybrid genotype, and some unidentified deficiency in hybrid dams (e.g. they may have produced smaller eggs) is the most likely explanation for this pattern. No significant differences were found among back-crosses grouped according to which species donated Y-chromosome/cytoplasm ($F = 0.27$, $p = 0.61$) or according to which species was the pure parent ($F = 0.24$, $p = 0.63$).

(*e*) *Sex ratio*

The reduction in F2 survivorship did not depend on gender as F2 broods showed no bias in sex ratio (*SR*, females: both sexes at eclosion = 0.47, $\Delta\log L = 0.12$, n.s.). Nor was there a bias in sex ratio for any other cross type: pure crosses, $SR = 0.49$, $\Delta\log L = 0.32$, n.s.; F1, $SR = 0.49$, $\Delta\log L = 0.22$, n.s.; BC-1, $SR = 0.45$, $\Delta\log L = 0.68$, n.s.; BC-2, $SR = 0.52$, $\Delta\log L = 0.39$, n.s.

4. DISCUSSION

Anartia fatima and *A. amathea* mated assortatively but there was no evidence of reinforcement (i.e. direct selection for assortative mating; Dobzhansky 1940) as homotypic mating frequency did not differ between allopatric and sympatric experiments. Thus, yet another hybrid zone study fails to support the reinforcement hypothesis (Butlin 1989; Barton & Gale 1993). The analysis of hybrid fitness revealed two examples of Haldane's rule (Haldane 1922). First, F1 hybrid females were partially sterile in both directions of the cross. Second, females from the *AF* cross (female *A. amathea* × male *A. fatima*) mated less frequently than other females (females from the *FA* cross-mated normally). The decreased mating propensity of *AF* females may have been caused by either low vigour or extreme choosiness (the rejection of all possible mates). Behavioural observations of *AF* female inactivity suggest the former, though the evidence remains anecdotal. The asymmetry of mating propensity suggests X-linked recessive genes, as inferior F1 hybrid females differ from healthy F1 hybrid females in the origin of their sex chromosomes. They also differ, however, in the origin of their cytoplasm and therefore maternal effects remain a possibility.

The reduced fitness of F1 hybrid females was limited to fertility and mating propensity. F1 larval survivorship tended towards heterosis and there was no bias in F1 sex ratio. This follows the pattern seen in *Drosophila* and mammals where F1 hybrid inviability (narrowly defined in terms of survivorship) evolves more slowly than Haldane's rule F1 hybrid male sterility (Wu 1992). Strong sexual selection on males, rather than Haldane's rule dominance theory, has been proposed to explain this bias (Wu *et al.* 1996). This 'special-male' hypothesis is not supported by the *Anartia* data, which instead suggest that the fertility of the heterogametic sex is more vulnerable to hybrid disruption. Further Lepidopteran examples are needed to establish the generality of this result.

In contrast to the high survival rate of F1 hybrids, F2 (F1 × F1) hybrid survivorship was only 15% of the normal. Survivorship in back-crosses (F1 × parental) was intermediate between that of F2 hybrids and parental broods. Sex ratios in F1 hybrids, F2 hybrids, and back-crosses did not differ significantly from unity, suggesting that Haldane's rule did not apply to survivorship. Dominance theory is consistent with this pattern of hybrid survivorship if it is mainly controlled by autosomal recessives, rather than sex-linked recessives (Turelli & Orr 1995). Under dominance theory, deleterious effects expressed in the heterogametic sex of F1 hybrids are caused by hemizygous X-linked recessives. Dominance theory assumes nothing special about X-linked genes, so deleterious effects are also expected due to interactions of homozygous autosomal recessives in both sexes of F2 hybrids (Turelli & Orr 1995). As butterflies have relatively small X-chromosomes, autosomal F2 hybrid breakdown should evolve quickly relative to F1 Haldane's rule. This is precisely the pattern in *Anartia*: F2 hybrid breakdown affects both sexes and is more severe than the F1 hybrids Haldane's rule effects on survival.

Some butterfly studies (Sperling 1994), however, are consistent with the 'special-X' theory, in which recessives on the X-chromosome evolve faster (Charlesworth *et al.* 1987). Grula & Taylor (1980*b*) traced X-chromosomes through the hybridization of the Pierid butterflies, *Colias philodice* and *C. eurytheme*.

They found a strong asymmetric Haldane effect (reduced female fertility and fecundity) in the F1 hybrids, which extended to back-cross and F2 hybrid females carrying the *C. philodice* X-chromosome. This is consistent with dominance theory, but many other differences (including mating preferences) between the two species were also X-linked (Grula & Taylor 1980*a*). When such small X-chromosome taxa have diverged enough to acquire many X-linked differences, dominance theory predicts that autosomal genes will already have caused substantial F2 hybrid breakdown—as in *Anartia*. F2 hybrids of *Colias*, however, were fertile and showed no significant decrease in survivorship. Only fecundity (of F2 males and females) appeared somewhat reduced.

If F2 hybrid breakdown evolves more slowly than F1 Haldane's rule in butterflies, dominance theory might need to incorporate some process causing faster evolution of X-linked genes (Rice 1984; Charlesworth *et al.* 1987; Hurst & Pomiankowski 1991). X-linked genes seem disproportionately important in differentiating species of Lepidoptera (Sperling 1994), and in *Drosophila* the X-chromosome has an important role in female sterility and inviability (Orr 1987; Orr & Coyne 1989; Sawamura 1996). These cases of X-linked hybrid inferiority in the homogametic sex must be flukes if there is no special-X effect.

The hybridization of *A. fatima* and *A. amathea* would not count as an example of Haldane's rule in most reviews (Coyne & Orr 1989*a*; Wu & Davis 1993), which usually require total sterility or mortality of one or both sexes. Hybrids were considered viable by Coyne & Orr (1989*a*) 'if any adults of that sex appeared, even rarely'. Such strict criteria eliminate populations with only mild hybrid abnormalities and risk excluding those at an early stage of speciation (Coyne 1992). The first incompatibilities in speciation may cause only slight hybrid inferiority, possibly expressed as poor ecological performance. This presents a problem for comparative studies because the ecological prowess of hybrids is extremely difficult to quantify and it is virtually impossible to compare it across diverse taxa. Our data suggest a way of overcoming this obstacle. We have presented a statistical method that allows mating preferences and mating propensities to be readily differentiated in multiple mate choice experiments. Not only does this permit a more accurate description of 'premating' isolation, but it may also reveal slight 'postmating' incompatibilities that reduce mating propensity. Such analyses are needed to evaluate the relative importance of assortative mating, Haldane's rule and F2 hybrid breakdown in speciation.

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APPENDIX 1. *Brood data*

cross type	totals					cross type	totals				
	eggs	larvae	males	females	eclosions		eggs	larvae	males	females	eclosions
		21	11	7	18		28	8	3	2	5
		34	11	21	32		105	4	1	0	
		151	52	47	99		171	94	20	13	33
		100	34	34	68		93	34	2	1	3
		0	0	0	0		105	42	8	8	16
	300	259	88	91	179		51	0	0	0	0
	333	190	77	73	150		36	4	1		2
	204	176	55	51	106		117	35	1	5	6
	6			0			55	28	6	3	9
	27	15	9	4	13		77	20	0	4	4
	23	20	4	9	13		60	35	7	5	12
	15	3		1	2		30	15	2	0	2
	34	34	3	5	8				0	0	0
	92	89	18	12	30		138	117	16	14	30
	108	42	12	15	27		20	18	8	5	13
	118	117	40	50	90		4	4		2	3
	81	56	14	11	25		13	13	8	4	12
	50	50	22	15	37		88	20	3	4	7
	9	3	2		3		44	18	3	4	7
	128	41	9	5	14		1		0	0	0
	290	192	64	53	117		31	6		2	3
	38	19	2	0	2		29	29	0		
	86	50	12	10	22		66	28	5	3	8
	130	105	27	31	58		116	97	37	52	89
	32	29	8	3	11		6	0	0	0	0
	39	32	0	0	0		87	76	32	34	66
	41	39	1		2		40	37	7	14	21
	459	425	141	143	284		83	60	22	29	51
	462	379	111	135	246		63	53	26	18	44
	40	34	19	5	24		23	17	3	5	8
	37	30	8	6	14		5	5	3	2	5
	180	117	57	43	100		3	3	2		3
	13	10	6	3	9		231	96	15	12	27
	215	152	50	45	95		173	43	5	6	11
	43	41	17	16	33		31	1	1	0	1
	26	25	10	2	12		18	15	2	2	4
	80	54	15	16	31		166	35	2		3
	6	6	2	4	6		297	212	8	7	15
	51	30	11	14	25		86	7	0	0	0
	41	34	8		9		26	8	1	1	2
	21	19	4	6	10		20	0	0	0	0
	41	38	4	4	8		13	7	0	0	0
	247	47	18	14	32		111	12		2	3