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Parasites increase fluctuating asymmetry of male *Drosophila nigrospiracula*: implications for sexual selection

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Abstract

Fluctuating asymmetry (minor deviations from perfect bilateral symmetry) is manifested by individuals less able to buffer environmental stress during development. I utilized a system of two naturally-occurring parasites of *Drosophila nigrospiracula* to test whether parasitic infection during host development yields elevated degrees of fluctuating asymmetry in two morphological traits of males. This hypothesis has important implications for sexual selection, as it may explain why asymmetric males are often found to be sexually disadvantaged. In my system, nematodes infect larvae and therefore are more likely to disrupt development than mites which only parasitize adult flies. As predicted, nematode-infected male *D. nigrospiracula* had a higher degree of bristle asymmetry than did mite-infested and control (carrying neither parasite) males. There was also a significant relation between nematode number and degree of asymmetry. There was a significant negative relation between nematode load and size of adult males, implicating a causal link between nutritional stress during host development and fluctuating asymmetry. Patterns of wing length asymmetry were inconsistent with those of bristle asymmetry. Nematode-infected males did not differ in wing length asymmetry relative to mite-infested and control males, nor was there a significant relation between nematode number and wing asymmetry. This inconsistency in expression of asymmetry may reflect different intensities of selection operating on each morphological trait.

Introduction

Bilaterally symmetrical traits of animals who experience ideal conditions during development are expected, in theory, to be expressed identically on the two sides of the body (Thoday, 1956; Soulé & Cuzin-Roudy, 1982). Perfect bilateral symmetry is expected because traits on the two sides are the product of the same genotype. Any random departure from perfect symmetry, therefore, has been interpreted to indicate reduced homeostasis or buffering of developmental processes (Thoday, 1956; Waddington, 1957). Fluctuating asymmetry (FA) is a measure of such random deviations from perfect bilateral symmetry in a morphological trait (Van Valen, 1962). Because FA may result from exposure to harmful environmental factors, such as chemical pollutants (Valentine, Soulé & Samallow, 1973), and/or from increased degrees of homozygosity (Soulé, 1982; Leary, Allendorf & Knudsen, 1984; Mitton & Grant, 1984; Leary & Allendorf, 1989; Clarke, 1992), it is, therefore, not surprising that high FA is often associated with reduced fitness at the individual level, and with greater probability of extinction at the population level (Leary & Allendorf, 1989).

High FA is also associated with reduced male reproductive success (RS) (Markow, 1987; Markow & Ricker, 1992; Thornhill, 1992). For example, Møller (1992a) found that male swallows (*Hirundo rustica*) who exhibited reduced FA in their outer-most tail feathers mated earlier in the breeding season and achieved greater annual reproductive success than less symmetrical birds. Møller (1992a) argued that females who discriminate in favour of low-FA males benefit by transmitting to their offspring genes that enhance viability. According to this hypothesis, males vary in the expression of FA in certain characters due to differential heritable ability to cope with environmental contingencies during development. This hypothesis may be subsumed under 'good genes' models of sexual selection, and is currently most often discussed by evolutionary biologists (Balmford & Thomas, 1992; Borgia & Wilkinson, 1992; Møller & Höglund, 1991; Møller, 1992a, b). However, the nature of the environmental stressor(s) which causes FA, and therefore the precise benefits accrued by FA-discriminating females, remain unidentified.

One possible stressor which could increase FA is inferior nutrition during development of a character, especially a character expressed at its physiological limits due to intense sexual selection. Alternatively, parasitic infection, perhaps exacerbated by malnutrition, may be causally related to FA. In fact, Bailit *et al.* (1970) suggest that poor nutrition and disease infection may increase FA in human dentition, and Livshits and Kobyliansky (1991) have suggested that maternal infectious diseases carried during pregnancy could account for high FA in infants.

In the present study I tested whether parasitic infection during development increases FA. Although the role of parasites in sexual selection is currently under intense study (reviewed by Clayton, 1991), any link between parasitic infection and FA is unidentified. A causal relationship between parasitic infection and high FA could be of considerable importance to the study of sexual selection because several hypotheses consequently arise that may explain why asymmetrical males suffer mating disadvantages, as mentioned above. These hypotheses will be discussed in a later section, but two that involve female choice are mentioned here. First, if the ability to resist parasitic infection has a genetic basis, as is often found to be the case (Steinhaus, 1949; Woodard & Fukuda, 1977; Petersen, 1978; Wakelin, 1985; Watanabe, 1987), females who choose symmetrical males as mates could benefit through transmission of resistance to offspring. This possibility parallels the Hamilton and Zuk (1982) hypothesis which has received considerable support, both from inter- and intra-specific studies (Read, 1987, 1988; Ward 1988; Clayton 1991; Zuk, 1991). According to this hypothesis, females discriminate in favour of unparasitized males on the basis of the degree of elaboration and coloration of secondary sexual characters.

According to a second female-choice hypothesis, females may prefer to mate with low-FA males because they gain direct benefits. For example, females may discriminate in favour of symmetrical males to avoid direct transmission of sexually-transmittable parasites to themselves and/or to offspring, or because unparasitized males are better able to provide quality territories or paternal care.

A fundamental assumption of these aforementioned hypotheses is that parasitic infection during development increases host FA. I tested this assumption by examining *Drosophila nigrospiracula* Patt. and Wheeler and two of its parasites, allantonematid nematodes and macrochelid mites. Both parasites co-occur with their drosophilid host in nature. Because nematodes infect *D. nigrospiracula* during its larval stages and mites infest adult flies only (see below), I expected that only nematodes could elevate host FA. Thus, I generated the following predictions from the hypothesis that parasitic infection during development can perturb development and increase FA. First, nematode-infected male *D. nigrospiracula* will exhibit greater FA than mite-infested and control (unparasitized by either parasite) flies sampled from the same population. In the event of a response to infection, I also predicted a positive relationship between the severity of infection and the magnitude of FA.

That both nematode- and mite-parasitized flies will exhibit greater FA than control flies is a prediction of an alternative hypothesis, namely that susceptibility to parasitization is a manifestation of inbreeding depression, and that individuals are more asymmetric because they are more homozygous (Reeve, 1960; Soulé, 1979; Mitton & Grant, 1984; Palmer & Strobeck, 1986). This alternative hypothesis, therefore, assumes no causal link between parasitic infection and FA.

Biology of nematodes

The infective life stage of an allantonematid nematode begins when a fertilized female nematode enters a fly larva. Entry is achieved by direct penetration of the cuticle with the aid of a spear-like stylet and associated pharyngeal gland (Poinar, 1983). These nematodes are nutritionally specialized parasites, and once inside their host, they feed from larval hemocoel by absorbing food directly through their body wall (Welch, 1959; Poinar, 1983). Nematodes achieve maximum size during host pupation at which stage they begin deposition of eggs into the host hemocoel (Poinar, 1983). Damage to flies due to nematode infection can be considerable. Fat bodies, especially those associated with the gonads, are reduced, and fewer eggs are produced by adult flies (Welch, 1959; Jaenike, 1992). For example, all female *D. putrida* infected by nematodes invariably suffer complete sterility (Jaenike, 1992). These findings suggest that nematodes compete with their host for nutritive material, and, as a consequence, the parasites may affect larval biochemical processes, disrupt development, and augment FA. Nutritional deficiencies, or the lack of certain constituents in the diet, are known to affect growth, development, and energy production in insect hosts (Steinhaus, 1949; Sang, 1956). In fact, reduced bioenergetic efficiency is associated with high FA in the sulfur butterfly, *Colias philodice* (Watt, Cassin & Swan, 1983; Leary & Allendorf, 1989).

Biology of mites

Macrocheles subbadius (Berlese) is a cosmopolitan mite associated with several dipteran species (G. W. Krantz, personal communication). Female mites attach to adult flies of both sexes by embedding the entire length of their chelicerae into the fly's abdominal tissue. This mode of attachment suggests that mites actually feed from the host hemocoel rather than assuming an entirely passive phoretic role, as generally believed. In fact, a laboratory study has shown that these mites significantly reduce longevity of both male and female *D. nigrospiracula*, and field work has demonstrated that attached mites have a marked effect on male mating success (Polak & Markow, manuscript in preparation). Thus, these mites exert intense selection on their adult hosts.

Materials and methods

Field collection of hosts

Drosophila nigrospiracula is endemic to the Sonoran desert of North America and breeds in necrotic saguaro cacti, *Carnegiea gigantea*. Male flies gather on the outside skin of these cacti where they court and mate with females who then oviposit in the rotting cactus tissue (Markow, 1988). Samples of male *D. nigrospiracula*, that included nematode-infected and mite-infested males, as well as males unparasitized by either nematodes or mites, were acquired by randomly collecting flies from necrotic saguaro cacti at five different sites within 40 mi of Phoenix, Arizona, USA, between 23 June and 22 October, 1992. Each site was visited 3 - 8 times. On each visit, approximately 25 males were collected from their mating arenas situated on the outer skin of the necrotic saguaro, gently aspirated into 8-dram vials containing banana-agar fly medium, and returned to the laboratory.

Scoring parasites and measuring FA

On the same day of collection, I anesthetized individual males in the laboratory using ether or CO_2 and counted mites attached to each male. Of all mites counted on males, 95% were found attached to the ventral surface of the abdomen near the junction with the thorax, although I occasionally found mites clinging to the neck or face of flies. After counting and removing mites, I measured thorax length of flies to estimate body size (Robertson & Reeve, 1952), removed the front coxae to expose bristles, and counted all bristles, including the sternopleurals, on the second preepisternum of each side of the body (Demerec, 1965). Bristles were counted using a Wild M3Z dissecting microscope. Sternal bristles were not counted. Bristle FA was calculated as the value of the right side minus the value on the left side of the body (R-L).

Using fine forceps, I carefully excised each wing and placed it on double-sided transparent tape adhered to a 35mm glass projection slide. Wings were firmly flattened against the tape using a clean piece of glass. Following the removal of wings, each fly was immersed into 2-3 drops of physiological saline (Shen's solution) on a depression slide and dissected to determine the number of mature nematodes in the body cavity. Dissections were performed under the Wild M3Z microscope. In saline, mature female nematodes, which are distinguishable by their conspicuous sausage-like shape, remained immobile and sank to the bottom of the depression slide. These females were commonly observed to contain eggs. When all dissections were completed, wing measurements were made on digitized images of magnified wings without knowledge of male infection status. The length measured was from the anterior crossvein to the junction of the second longitudinal vein with the outside margin of the wing. Three repeated measures were made of each wing using microcomputer

Optimas software (BioScan Inc.). As for bristle FA, wing-length FA was calculated as the wing length value on the right minus the value on the left (R-L). Because wing length was strongly related to thorax length (slope = 35.6, $F_{(1,\ 159)} = 259.2$, $P = 0.0001$, $R^2 = 0.63$), I used wing length as an estimator of male body size throughout the study.

Three of the males dissected contained hundreds of larval nematodes distinguished by their thread-like appearance and sustained whip-like movements. Because I was unable to identify mature nematodes among the writhing mass of juveniles, measurements made on these three males were not included in analyses requiring exact counts of female nematodes. In addition, occasional samples (N = 4) were comprised exclusively of males uninfected by nematodes. Measurements made on males from these samples were excluded from all statistical analyses. Males (N = 2) that were parasitized by both types of parasite were also discarded.

Statistical analyses

Van Valen (1962) distinguishes three modes of asymmetry, directional asymmetry (DA), antisymmetry (AS), and fluctuating asymmetry (FA). DA and AS are products of genetical or developmental processes, while FA reflects environmentally-induced perturbations that occur during an organism's development. Thus, in order to evaluate true FA, one must obtain a measure independent of DA and AS. For a trait exhibiting true FA, variation of signed (R-L) values should be normally distributed with a mean equalling zero (Van Valen, 1962; Palmer & Strobeck, 1992). Departure from mean zero identifies DA, while non-normal distributions may reflect several population phenomena (Palmer & Strobeck, 1992), including the presence of AS (handedness) (e.g. Yamaguchi, 1977). To evaluate whether each trait used in this study exhibited true FA, I calculated means, 95% confidence intervals of means, skewness, and kurtosis for signed (R-L) values of each trait.

Another factor confounding analyses of asymmetry is the presence of an association between the magnitude of a trait and the degree of asymmetry (Palmer & Strobeck, 1986). Thus, in order to detect size dependence in bristle and wing length FA, I regressed trait asymmetry (absolute value of R-L) on the magnitude of each trait. In cases where size dependence was found, I scaled out the effect of size by dividing FA by the average size of the trait (R+L/2).

To assess difference in bristle FA among the three experimental groups of males, I conducted a Kruskal-Wallis test on absolute values of (R-L) calculated separately for each male. Wing length FA for each group was obtained from a two-way analysis of variance in which 'side of body' is one factor (fixed) and males the other (random) (Palmer & Strobeck, 1986). Because three repeated measurements of each wing were taken in this study, this analysis allows the variance due to measurement error to be subtracted from the interaction (sides × males) variance. The resultant variance, divided by three (number of replicate measures made on each wing, was the estimate of FA for each male group. The Bartlett's and F_{MAX} tests for homogeneity of variances were used to assess differences among groups of males. As an alternative method of distinguishing FA among groups, I calculated wing length FA as the absolute value of (mean R - mean L) for each male within groups, and tested for group differences by Kruskal-Wallis analysis of variance.

To determine whether there was an association between parasite load and FA of each trait, I performed regression analyses of scaled FA on parasite number. Assumptions of these analyses were evaluated by inspecting plots of the residuals versus the independent variable (FA) (Neter, Wasserman & Kutner, 1990).

Results

Mean parasite loads of those males that were parasitized were 1.9 nematodes (SE = 0.24, N = 24, range 1-5) and 3.1 mites (SE = 0.32, N = 51, range 1-10). Mean bristle number and mean wing length for each group of males are presented in Table 1. From one-way ANOVA, neither mean bristle number ($F_{(2,\ 199)} = 0.62$, $P = 0.54$) nor mean wing length ($F_{(2,\ 175)} = 0.22$, $P = 0.81$) was significantly different among the three groups of males (Table 1). However, bristle number showed a two-fold greater coefficient of variation than wing length, and values were repeatable among groups (Table 1). These coefficients are independent of the mean and thus serve to compare relative amounts of variation between traits (Sokal & Rohlf, 1981).

Table 1. Means (SE, N) and coefficients of variation (CV) in both bristle number and wing length measures of parasitized and control (uninfected) groups of male *Drosophila nigrospiracula*. FA represents absolute values of R-L.

	Nematodes	Mites	Control	Pooled sample
Bristles				
Per side	18.13 (0.40, 27)	18.51 (0.29, 51)	18.63 (0.19, 124)	18.55 (0.147, 202)
CV (per side)	11.44	11.34	11.35	11.31
FA	2.36 (0.26, 27)	1.29 (0.171, 51)	1.45 (0.108, 124)	1.54 (0.87, 202)
Wings				
Length (mm)	1.56 (0.02, 24)	1.58 (0.015, 44)	1.58 (0.08, 109)	1.58 (0.007, 177)
CV (length)	6.12	6.19	5.21	5.57
FA	0.012 (0.001, 24)	0.011 (0.001, 44)	0.011 (0.001, 109)	0.011 (0.001, 177)

Bristle number and wing length FA met the requirements of reliable indicators of developmental instability (Palmer & Strobeck, 1992). For pooled samples of males, the 95% confidence interval of each (R - L) mean included zero, and no significant skewness was detected in the (R - L) distribution of either trait (Table 2). Although bristle FA did not exhibit kurtosis, wing asymmetry was significantly leptokurtic (Table 2). Leptokurtosis indicates that fewer values fall within intermediate regions relative to a normal distribution (Sokal & Rohlf, 1981).

There was no relation between the magnitude of bristle FA and character size (bristles per side) (slope = 0.08, $F_{(1, 200)}$ = 0.40, P = 0.53), nor was there a relation between bristle FA and male thorax length (slope = - 0.004, $F_{(1, 178)}$ = 0.02, P = 0.88). However, wing length FA exhibited some size dependence; there was a negative and marginally insignificant relation between wing length FA and wing length (slope = - 0.03, $F_{(1, 176)}$ = 3.61, P = 0.058).

There was a significant negative relationship between male thorax length and nematode load (Fig. 1), suggesting that nematodes make considerable nutritional demands during larval development of flies (the relation between thorax length and mite load was insignificant (slope = - 0.22, $F_{(1, 19)}$ = 0.72, P = 0.40). I tested whether fluctuating asymmetry in the two characters was greater in nematode-infested males than in mite-infested and control (unparasitized) males. As predicted, bristle FA was significantly greater among nematode-infected males than among mite-infested and control males

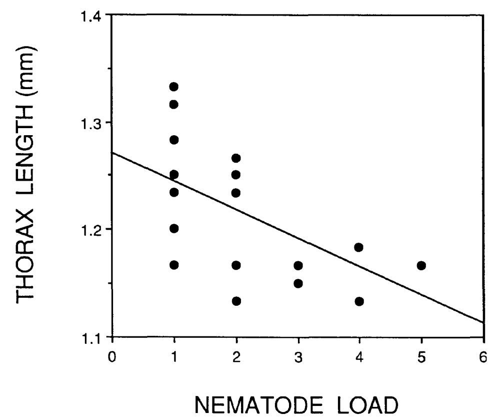

Fig. 1. Negative relationship between thorax length and the number of mature nematodes found within male *D. nigrospiracula*

Table 2. Mean, 95% confidence interval (CI), skewness and kurtosis in bristle number and wing length asymmetry (signed R-L) of male *Drosophila nigrospiracula*.

Trait	Mean FA	N	95% CI	Skewness (P)	Kurtosis (P)
Bristles	0.144	202	-0.130, 0.417	0.037 (> 0.5)	-0.264 (> 0.2)
Wing length	-0.002	177	-0.004, 0.0001	0.008 (> 0.5)	1.29 (< 0.001)

(Fig. 2). Moreover, the response of FA to nematode infection was dose-dependent; among males harboring nematodes, scaled bristle FA was significantly related to the number of mature nematodes within flies (Fig. 3), and, as predicted, scaled FA was unrelated to the number of attached mites (Table 3, Fig. 3).

Results of two-way analyses of variance computed on wing length asymmetry are shown in

Fig. 2. Mean absolute FA values for thoracic bristles in three groups of male *D. nigrospiracula*. Nematode-infected males were significantly more asymmetric than mite-infested and control (unparasitized) males (Kruskal-Wallis H = 13.29, P = 0.0013). Means are presented in Table 1. Sample sizes are provided in the figure.

Table 3. Results of regression analyses of bristle number and wing length FA (scaled for character size) on nematode and mite loads in male *Drosophila nigrospiracula.*

	Slope	F (df)	P	R^2
Bristle FA				
nematodes	0.03	5.46 (1, 20)	0.03	0.21
mites	0.003	0.56 (1, 49)	0.48	0.01
Wing length FA				
nematodes	-0.002	0.09 (1, 20)	0.76	0.01
mites	-0.001	0.35 (1, 42)	0.55	0.01

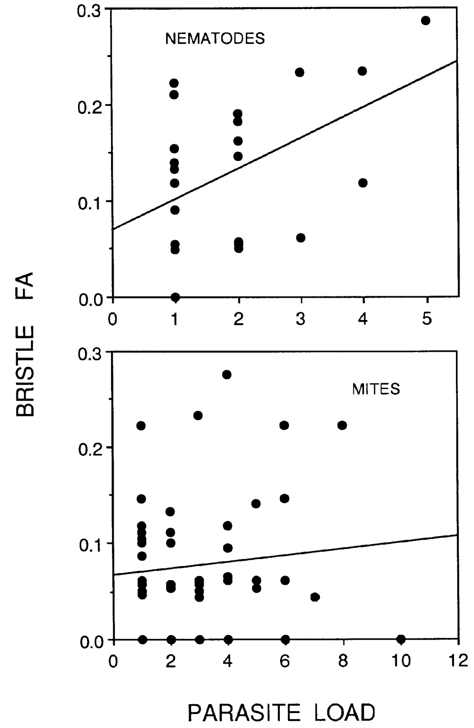

Fig. 3. Relationship between absolute value of bristle FA and number of nematodes and mites parasitizing male *D. nigrospiracula.*

Table 4. Mean squares (ms, × 10^5) from two-way analyses of variance of wing length values from parasitized and control groups of male *Drosophila nigrospiracula*. These means squares were used to calculate the error ms as a percentage of interaction ms (non-directional asymmetry), and the variance component representing FA.

	Error	Interaction	Error/Interaction (× 100)	Fa*	df**
Nematodes	0.29	24.84	1.2%	8.18	40
Mites	0.34	31.81	1.1%	10.49	42
Control	0.31	28.67	1.1%	9.45	106

* Bartletts's and F_{max} tests for homogeneity among FA variances, B = 0.544, 0.90 < P < 0.95, and F = 1.28, P > 0.05, respectively.

** Degrees of freedom associated with FA calculated by the Satterthwaite formula.

Table 4. No difference in FA among the three groups was detected by either the Bartlett's or F_{MAX} test (Table 4). Because the relative contributions of measurement error were small relative to FA for each male group (< 2% for each group, Table 4), I re-calculated FA as the absolute value of (mean R - mean L) for each male, and scaled these values to uncouple FA from character size. Consistent with two-way ANOVA, there was no difference in scaled FA among groups (Kruskal-Wallis H = 2.38, P = 0.30). Among males parasitized by nematodes and mites, there was no relation between scaled wing length FA and severity of parasitization by either parasite (Table 3).

Discussion

Parasites, FA and sexual selection

Results of the present study support the novel hypothesis that parasitic infection during host development augments host developmental instability, and hence FA. Bristle FA was highest among male *D. nigrospiracula* infected with allantonematid nematodes relative to individuals burdened with an adult-infesting mite and unparasitized by either nematodes or mites. Moreover, FA in males was dose-dependent; there was a significant relation between nematode number and the magnitude of FA. Below, I discuss how my findings may be used to explain why asymmetrical males of certain species suffer reduced reproductive success.

One alternative to the hypothesis of a causal mechanism connecting elevated FA with parasitism is that individual flies harbor more parasites due to inbreeding depression and are therefore also more asymmetrical. However, there are two lines of evidence that cast doubt on this alternative. First, there was no relation between FA and the probability of mite infestation, and second, two-generation inbred (sib-mated) *D. nigrospiracula* did not exhibit higher bristle FA than outbred, control lines of flies (Polak, unpublished data).

There are two lines of evidence that make it reasonable to expect that, in *D. nigrospiracula*, low FA indicates heritable ability to resist parasitic infection. First, in some drosophilids, infection by allantonematid nematodes can reduce male mating success and female fertility to the point of complete sterility (Jaenike, 1988, 1992). These results are important, because together with an inverse relationship between nematode number and adult host size found in the present study, they suggest that nematodes represent a strong agent of selection in *Drosophila*. Such strong selective pressures make it reasonable to expect an evolved host response against infection. In fact, response by *Drosophila* to nematode infection is complex and includes melanization, encapsulation, and expulsion (Welch, 1963). Furthermore, that resistance arises in populations of other species under selection against pathogens and even insecticides is well documented (Keiding, 1977; Mulla, 1977; Petersen, 1978; Davidson, 1992). For example, within approximately 104 generations of laboratory selection in mosquito colonies, behavioral changes arose that enabled better active defense by larvae against penetration by mermithid nematodes (Woodard & Fukuda, 1977). In selection experiments with the silkworm, *Bombyx mori*, against a cytoplasmic virus, Aizawa, Foruta and Nakamura (1966) found resistance in only 13 generations. In a case involving chemical insecticides, resistance to dieldrin arose in natural populations of the Australian sheep blowfly, *Lucilia cuprina*, within two years of its introduction (Clarke & McKenzie, 1987).

Second, a field study on the prevalence of infection showed that the distribution of nematodes was more aggregated among drosophilid flies than that expected under a random distribution (Jaenike, 1992). This finding can be interpreted as evidence for genetic heterogeneity in susceptibility (Anderson & Gordon, 1982). Also significant is the presence of *Drosophila* parasitized by allantonematid nematodes found in 26 million year old Dominican amber (Poinar, 1984). This finding certainly suggests a prolonged co-evolutionary history between these animals and presents the possibility that coadaptational cycles have occurred, which could maintain heritable variation for resistance (Clarke, 1979; Hamilton, 1982; Hamilton & Zuk, 1982). In conclusion, because high FA in male *D. nigrospiracula* is associated with nematode infection, and because it is possible that a genetic basis to resistance exists, low FA may reflect a heritable ability to resist parasitic infection.

Although high FA is associated with reduced male reproductive success in several species (Møller, 1992a; Thornhill, 1992), including *Drosophila* (Markow, 1987; Markow & Ricker, 1992), the mechanism(s) responsible for this mating pat-

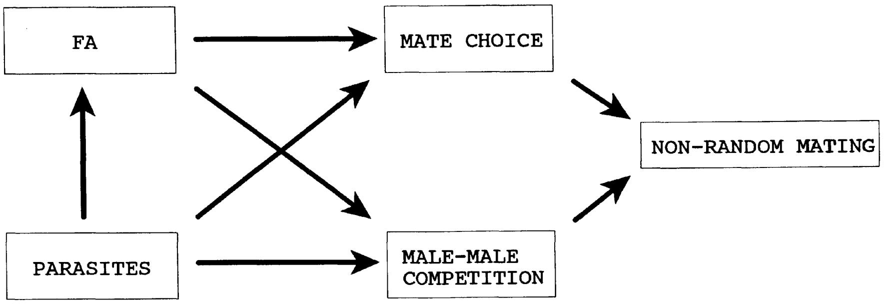

Fig. 4. Flow diagram illustrating how parasite-induced FA may effect non-random mating through mate choice by females and intra-male competition. Under a null model, FA is simply an incidental response to infection and non-random mating is due to parasitism only. Alternatively, FA may be used directly by females to distinguish resistant males or FA may reduce the ability of males to win intra-sexual contests over females. Finally, high FA and parasitism may interact to influcence host sexual selection. See text for further details.

tern remain unidentified and even controversial (Balmford & Thomas, 1992; Borgia & Wilkinson, 1992). One of the most popular hypotheses to explain this mating bias is female choice in favor of symmetrical mates (Møller, 1990, 1992a; Møller & Höglund, 1991; Thornhill 1992). According to this hypothesis, females choose symmetrical mates because symmetry reflects some form of heritable male quality that affects offspring viability. Female choice for low-FA males may thus be subsumed under 'good genes' models of sexual selection (Trivers, 1972; Heisler, 1984; Kodrick-Brown & Brown, 1984; reviewed by Kirkpatrick & Ryan, 1991). Because it is reasonable to apply my findings to other species, I suggest that low-FA males possess greater resistance against parasites, and that females may use observed differences in FA as indicators of male quality, specifically as it relates to male ability to resist parasitic infection. By choosing to mate with low-FA males, females may be more likely to produce parasite-resistant offspring. Hamilton and Zuk (1982) proposed a similar hypothesis, according to which only healthy males can develop the most elaborate and most brightly colored secondary sexual characters. Discriminating females prefer the showiest traits because those males able to produce them are resistant to parasitic infection.

What evidence exists for female mate choice in favor of symmetrical males? Møller's (1992a) experiments, which consisted of manipulating tail length asymmetry of male swallows (*Hirundo rustica*), showed that males with symmetrical tails acquired mates sooner in the breeding season and had greater annual reproductive success than asymmetric males. In the Japanese scorpionfly, *Panorpa japonica*, Thornhill (1992) documented female preference for the pheromone of symmetrical males over less symmetrical individuals. In *P. japonica*, however, it is not known whether females accrue material or genetic benefits from mating with symmetrical males; low-FA males also tend to be more successful at acquiring quality food items on which females feed during mating (Thornhill, 1992).

Although active mate choice for symmetrical mates is currently most often discussed, Fig. 4 illustrates other ways by which parasites and FA may influence host sexual selection. Under a null model, FA is simply an incidental response to parasitism and has no influence on host sexual selection. According to this hypothesis, negative effects of parasites alone account for observed non-random mating of hosts. Such effects are legion. For example, parasites may reduce male attractiveness through their affect on male sexual secondary characters (Hamilton & Zuk, 1982; Read, 1987, 1988; Ward, 1988; Houde & Torio, 1992), courtship display (Kennedy *et al.*, 1987), or because parasites themselves are detected by females (Borgia & Collis, 1989; Spurrier, Boyce & Manly, 1991). Parasites may reduce male competitive ability or disrup dominance hierarchies (Howard & Minchella 1990), cause their host to voluntarily reduce curren reproductive effort (Forbes, 1992), and change hos behaviour to facilitate parasite transmission (Moore, 1984; Edwards & Barnard, 1987).

Alternatively, FA may be used directly by females to discriminate in favour of healthy, unparasitized males, as already discussed (but see below). Non-random mating may also occur if asymmetry weaponry, or characters important for maneuverability, reduce male ability to win intra-sexual contests over females. Finally, the effects of high FA and parasitism may interact, and hence simultaneously influence host non-random mating.

Intensity of selection and FA

Although bristle FA was correlated with nematode load, wing length FA was not. Wing length was, however, associated with a two-fold smaller coefficient or variation than bristle number. This finding has several important implications. First, since stabilizing selection can be expected to decrease the variance of the frequency distribution of a trait (Soulé, 1982; Endler, 1986), these contrasting values of variation suggest that wing length may be under stronger stabilizing selection relative to bristle number. Second, because coefficients of variation are useful also in estimating susceptibility of characters to developmental instability (Mitton, 1978; King, 1985), bristle number is probably less canalized during development relative to wing length. For example, Eanes (1978) showed that heterozygosity in the monarch butterfly is inversely related to morphological variability, due to an heterozygote advantage to canalize morphology during development. Thus, the finding in *D. nigrospiracula* that wing asymmetry was insensitive to parasite-induced developmental stress may be the result of especially strong selection favouring effective wing canalization during development.

The life history of *D. nigrospiracula* requires that individuals disperse long distances to track suitable necrotic cacti, and asymmetric wings may impede effective long-distance dispersal. The strength of selection is probably greater during summer months, when high temperatures, low substrate density and rapid substrate desiccation require continued dispersal attempts of up to 1 km per day (Johnston & Heed, 1976). Thus, selection for wing length symmetry is expected to be strong because asymmetric males may not survive the flight to fresh substrates. This hypothesis is supported by the finding that in *D. mojavensis*, another desert endemic *Drosophila* which feeds on organ-pipe cactus, mated males did not exhibit greater wing length FA than unmated flies (Markow & Ricker, 1992). However, in *D. pseudoobscura*, a widespread generalist species (Begon, 1982; Lakovaara & Saura, 1982), mated males did exhibit greater wing FA than single individuals (Markow & Ricker, 1992).

In the present study, there was a negative relation between absolute wing length asymmetry and wing length. This is a surprising finding because larger individuals should be more asymmetrical for simple allometric reasons and because phenotypically extreme individuals are expected to be more homozygous at the loci controlling body size (Soulé, 1982; Soulé & Cuzin-Roudy, 1982). This pattern of asymmetry is explained, however, if costs of asymmetric wings increase with body size (wing loading), a prediction from the hypothesis that asymmetry impedes effective flight. Research on the aerodynamics of avian fligth shows that asymmetry in tail feathers reduces lift, maneuverability and prey-catching ability of birds (Møller, 1991; and see Balmford & Thomas, 1992).

In summary, my data suggest that traits under very strong stabilizing selection express less observable FA. This idea is supported by two comparative studies showing that traits such as wing length exhibit less FA than which are apparently under directional selection due to either female choice or male-male competition (Møller & Höglund, 1991; Møller, 1992c). These findings imply that traits under strong stabilizing selection may be less useful to FA-discriminating females than traits under weaker stabilizing, or directional selection. In fact, in modelling the origin of female mating preferences, Heisler (1984) showed that traits under very strong (and very weak) stabilizing selection are least likely to be targets of female choice.

Another consideration is whether FA could ever be the focus of adaptive female choice; the process of either stabilizing natural selection operating on a trait and/or female choice operating on FA should cause rapid trait canalization and reduce asymmetry to dimensions unobservable by females. Thus, the nature and strength of selection, and hence the degree of trait canalization, are important variables that should be embraced by future hypotheses regarding the evolution and maintenance of adaptive mate choice on the basis of fluctuating asymmetry.

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