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Lack of evolutionary potential of developmental instability of front tibia length in the Indian meal moth (*Plodia interpunctella*)

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ABSTRACT. The evolutionary potential of developmental instability (DI, defined as an individual's inability to buffer its development against random perturbations) as estimated by individual asymmetry (so-called fluctuating asymmetry, small random deviations from perfect symmetry), remains a controversial subject of research. Only if DI is heritable and if it is related with fitness, can evolution be expected to occur. In this study we find no evidence for evolutionary potential of DI of the front tibia in the Indian meal moth. The heritabilities of fluctuating asymmetry and DI were negative. Individual asymmetry was not related to two fitness components: survival probability and body length. In contrast, both survival probability and body length were heritable. Body length increased with food quality but was not affected by density, whereas survival probability increased with food quality and decreased with density. Thus, effects of environmental stress and genetic background differed for fluctuating asymmetry on one hand and survival probability and body length on the other hand.

KEY WORDS: developmental instability, fluctuating asymmetry, fitness, heritability, Indian meal moth, *Plodia interpunctella.*

INTRODUCTION

The development of an individual is disturbed by random perturbations. As a consequence, its phenotype deviates from the expected one given the individual's genotype and the environmental conditions under which it developed. It is generally assumed that control mechanisms exist that buffer development against these mistakes (e.g. VAN DONGEN et al., 1999c). Developmental instability is the inability of an individual to buffer its development against these random perturbations (PALMER & STROBECK, 1992) [further called DI]. A commonly used estimator of DI is fluctuating asymmetry [i.e. small random deviations from perfect symmetry (Van Valen, 1962) further called FA]. The idea is that when trait development is perfectly buffered against perturbations, both sides of a bilaterally symmetric trait will undergo identical developmental pathways, since they are under the control of the same genes and environmental conditions, resulting in a perfectly symmetrical trait. Yet, development is never free of mistakes and perfect symmetry does not exist in nature (SWADDLE & CUTHILL, 1997). The poorer the development is buffered (i.e. high DI), the stronger deviations from symmetry are expected (i.e. high FA). DI and FA are assumed to originate from environmental and genetic effects as well as from genotype×environment interactions (MARKOW, 1995). The relative importance of these three factors, however, remains unclear, although it is critical for the interpretation of patterns in fluctuating asymmetry. The recent boost in studies of FA as estimator of quality, health or the effects of heterozygosity of individuals and populations (MARKOW, 1995; Leung & Forbes, 1996) has provoked much interest in the genetic architecture and evolutionary potential of DI (VAN DONGEN & LENS, 2000a; VAN DONGEN, 2000).

If fitness relates to particular phenotypic values, individuals that approximate these values with highest precision during ontogeny, and thus have low expected levels of DI, might have a fitness advantage (FOWLER & WHITLOCK, 1994). Furthermore, individual asymmetry might signal individual genetic quality and be used as a cue in mate selection under the 'good genes' model of sexual selection. Both the extents to which DI reflects

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individual quality and expected fitness, and the genetic variance of DI have important implications for evolutionary and sexual selection theory (DUFOUR & WEATHERHEAD, 1996). Developmental instability will be important for the genetic structure and evolutionary potential of populations only if it is related to the genotype and if it is associated with fitness (MARKOW, 1995). However, both the heritability of FA (h^2_{FA}) and DI (h^2_{DI}), as well as the relationship between DI and fitness remain controversial topics.

Recently, MØLLER & THORNHILL (1997) performed a meta-analysis to combine all available estimates of h^2_{FA}. They concluded that there is on average a small but highly significant genetic component (h^2_{FA}=0.19). However, their study has been criticized by several authors, many of them arguing that h^2_{FA} is much lower (<0.1) or even zero (LEAMY, 1997; MARKOW & CLARKE, 1997; WHITLOCK & FOWLER, 1997). Recent studies not included in the Møller and Thornhill paper seem to confirm this (VAN DONGEN et al., 1999a; VAN DONGEN, 2000 and references therein). WHITLOCK (1996) and HOULE (1997) noted that h^2_{FA} is expected to be much lower than h^2_{DI} because of the low correlation between individual asymmetry and DI. WHITLOCK (1996) [see also VAN DONGEN (1998a) and WHITLOCK (1998) for a correction of the formula] derived a way based on the hypothetical repeatability (*R*) of individual asymmetry, to transform h^2_{FA} into h^2_{DI}. This value of R estimates the proportion of variation in asymmetry that is the result of between-individual differences in the underlying DI. R, therefore, estimates how accurately individual asymmetry estimates individual DI. Similarly, GANGESTAD & THORNHILL (1999) argued that h^2_{FA} underestimates h^2_{DI} whereby h^2_{DI} may be in the order of magnitude found for many other fitness traits (i.e. h^2_{DI}=0.35-0.55). In agreement, LEAMY (1999) found a low heritability of FA (mean h^2_{FA}=0.03) but a much higher estimate for DI (mean h^2_{DI}=0.45), although neither estimates was statistically significant. Two other recent studies show that h^2_{DI} may be low as well [mean h^2_{DI}=0.09 in *Operophtera brumata* (VAN DONGEN et al., 1999a) and mean h^2_{DI}=0.04 in *Drosophila melanogaster* (WOODS et al., 1998)].

The relationship between DI and fitness is also not ubiquitous. LEUNG & FORBES (1996) performed a meta-analysis and concluded that FA-fitness relationships are fairly weak and highly heterogeneous. As for h^2_{FA}, the low correlation between individual asymmetry and DI may have obscured patterns in some studies (GANGESTAD & THORNHILL, 1999). Nevertheless, in the winter moth, VAN DONGEN et al. (1999a, 1999d) showed that fitness is not correlated with individual FA despite high values of *R*. Furthermore, individual asymmetry does not necessarily correlate with all fitness components (e.g. UENO, 1994; VAN DONGEN et al., 1999d).

As yet there is no theoretical framework that predicts under which conditions FA is expected to provide a reliable estimate of individual or population fitness, '(genetic) quality' or 'health'. This seriously hampers the general use of FA (BJORKSTEN et al., 2000) and calls for more detailed experiments (VAN DONGEN & LENS, 2000b). Investigating the evolutionary potential requires information on both the heritabilities of FA and DI as well as their relationships with fitness. Therefore, we estimate h^2_{FA}, h^2_{DI}, and the relationship between DI and fitness simultaneously for the Indian meal moth (*Plodia interpunctella*). In addition, we study two fitness traits. We performed full-sib breeding experiment estimating the effect of genotypic and environmental (density and food quality) factors on fitness and asymmetry. In this way we determined the relative contributions of environment and genotype to variation in FA, which allows us to evaluate the applicability of FA as an estimate of individual and/or population level 'quality'. Statistical power is an important, yet mostly ignored, issue in studies of FA. We propose a simulation approach to determine maximal values of h^2_{FA} and h^2_{DI}, and maximal effects of environmental conditions on population level FA.

MATERIAL AND METHODS

Breeding experiment

Thirty virgin females were randomly paired with 30 virgin males for copulation. Females were allowed to lay eggs on a feeding medium that consisted of wheatflour (77%), yeast (15%) and glycerol (8%) (further called OPTIMAL). Small, recently hatched caterpillars were transferred to experimental jars containing 2g of food. This food was freshly prepared and not replaced during the development of the caterpillars. The food did not show any obvious signs of decay during the growth period of the caterpillars. Three types of food were used: OPTIMAL, WHEAT (wheatflour only), and POTATO (potato meal only). Caterpillars were placed at three different densities: 5, 10 and 20. Thus, progeny of each family were partitioned over nine jars corresponding to the different food quality - density combinations. These treatments could not be replicated within families because number of offspring per female was too low. Both WHEAT and POTATO diets are expected to cause nutritional stress (GAGE & COOK, 1994). Yeast forms an important protein source in the artificial diet. In addition, potato meal contains nearly zero protein and fat resources. The relatively high densities are expected to increase nutritional stress even further.

Emerging moths were stored at -80°C and were sexed and measured afterwards. For the offspring of 20 families we measured body length to the nearest 0.1 mm, and determined length of the tibia of the front legs twice to the nearest 0.033 mm as outlined in detail in VAN DONGEN et al. (1999a; 1999c). We carried out an initial screening of different traits (the tibias of all legs and the length of a wing vein), hoping to be able to analyse different traits as suggested by SWADDLE (1997). However, this was not

possible because the development of the different legs was interdependent (VAN DONGEN et al., 1999c) and wing vein length could not be measured accurately. Without a thorough understanding of the mechanisms of this correlated development, a multivariate analysis of the genetic background of FA and DI of different traits could be misleading. Survival was expressed as the proportion of caterpillars surviving to the adult stage in the different family-by-treatment combinations.

Body length (i.e. length from the top of the head to the tip of the abdomen) is used as an indirect measure of fecundity and mating success (PODOLER, 1974; GREENFIELD, 1982; GAGE & COOK, 1994). If individual asymmetry is related to fitness in the Indian meal moth, we predict that population level FA should be highest in the treatments that exhibit the lowest survival during development, while individual asymmetry and body length should be inversely correlated. If FA is size-dependent, a positive association is expected.

Statistical analysis

Fluctuating asymmetry

We separated real FA from measurement error (ME) by mixed regression (VAN DONGEN et al., 1999b). The distribution of the signed asymmetry (left minus right trait value averaged over the two within-subject repeats) was tested for normality (using the Shapiro-Wilks' statistic, NETER et al., 1990) and for kurtosis in particular (following SOKAL & ROHLF, 1995). A positive kurtosis is expected when individuals differ in their underlying DI since the signed asymmetry is a mixture of different normal distributions with zero means and different variances (VAN DONGEN, 1998A; 1998B; GANGESTAD & THORNHILL, 1999). The hypothetical repeatability of individual asymmetry was obtained following VAN DONGEN (1998a). Data from all treatments and from both sexes were pooled, because this reveals the maximal degree of variation in DI. Individual asymmetry was estimated as the unsigned asymmetry. These values were used for subsequent analyses.

Genetic and environmental effects

Survival probability, body length, tibia length, and individual tibia asymmetry were analyzed by mixed model ANOVA. Each individual observation was entered in the analysis. Food quality, density and sex were treated as fixed effects, whereas family and its interaction with food quality and density were treated as random effects. The fixed-effects model the overall differences averaged across all families. Variance components of the random effects estimate genetic variance and genotype×environment interactions (where different genotypes respond to the environment in different ways, LYNCH & WALSH, 1997) respectively. The three-way random-effects interaction (i.e. family×food-quality×density) was added to the model as well. This variance component is confounded with common environment effects, because the individual food-by-density combinations were not replicated within families. However, by adding it to the model, the other variance components become unconfounded because possible common environment effects are explicitly modeled (unpublished simulations by S. Van Dongen). Significance of variance components was tested by likelihood ratio test and fixed effects were tested by traditional F-tests, adjusting the denominator degrees of freedom by Satterthwaite's procedure (details in VERBEKE & MOLENBERGHS, 1997). Heritabilities were calculated as twice the variance between families divided by the total variance (FALCONER & MACKAY, 1996). All analyses were performed in SAS (version 6.12). Body length, tibia length and individual unsigned asymmetry (the latter after logarithmic transformation) were nearly normally distributed (Shapiro Wilks' $W>0.95$) and were analyzed with PROC MIXED in SAS, assuming normality of the error terms. Survival probability was analyzed with the macro GLIMMIX, using logit link function and binomial error structure (e.g. CRAWLEY, 1993). This macro uses a quasi-likelihood approach (WEDDERBURN, 1974), can be obtained from the SAS server (http://www.sas.com/) and is described in LITTELL et al. (1996).

Power considerations

Because individual asymmetry is only weakly correlated with the underlying DI, expected heritabilities are small (WHITLOCK, 1996; HOULE, 1997; GANGESTAD & THORNHILL, 1999). Therefore, statistical power will be low and needs to be considered when negative results are found. In particular, we determined the maximal value of h^2_{DI} that could have led to the observed heritability. This can be done by generating data under a variety of alternative hypotheses (i.e. $h^2_{DI}>0$) and testing if this positive heritability is significantly larger than the observed value. We simulated data with a hypothetical repeatability and degree of FA equal to the observed values under a range of values for h^2_{DI} and determined the probability of obtaining the observed result just by chance (see below for details). If this probability was smaller than 5%, h^2_{DI} was considered significantly larger than the observed heritability. The level of h^2_{DI} for which this probability equals 5% is the maximal value. All simulations were performed in SAS (version 6.12) and the probabilities were determined from samples of 10,000 simulations.

Population level FA is expressed as a variance (or the average of the individual unsigned asymmetry values, which is equivalent, Whitlock, 1996). The estimation of a variance is subjected to large sampling variation, and the statistical comparison of different variances has low statistical power (PALMER, 1996; VAN DONGEN, 1999a). Therefore, we performed simulations in SAS to determine the maximal difference in population level FA between the different treatments (see results section for details).

RESULTS

Fluctuating asymmetry

Mixed regression analysis showed that 96% of the variation in tibia length reflected between-individual variation (σ^2=0.145). Real asymmetry explained 3% of the total variation (σ^2=0.004) and was significantly different from zero (χ^2_1=243, p<0.0001). The remaining 1% of variation (σ^2=0.002) reflected measurement error. There was no indication of directional asymmetry ($F_{1,431}$=0.34, p=0.6). The signed FA was nearly normally distributed (W=0.98), but had a small, yet statistically significant positive kurtosis (kurtosis=1.6, SE=0.23, t_{430}=6.8, p<0.001), indicating significant between-individual heterogeneity in the presumed underlying DI. The hypothetical repeatability of individual single trait asymmetry equaled 0.2.

Genetic and environmental effects

Survival probability decreased significantly with increasing densities and decreasing food quality, whereas body length decreased significantly with food quality only (Table 1, Fig. 1). In both cases, there was no interaction between the effects of food quality and density. Females were significantly larger than males. Individual unsigned asymmetry (after log transformation) did not differ between the different treatments or between males and females (Table 1, Fig.1).

TABLE 1

Overview of tests of fixed and random effects of the mixed ANOVA models for survival, body length, tibia length and log-transformed tibia unsigned asymmetry. (*: p<0.05; **: p<0.01; ***: p<0.001; significant effects are indicated in bold)

	Survival	Body length	Tibia length	Tibia unsigned FA
Source				
FIXED FACTORS				
Density	$\mathbf{F_{2,171}=8.3}^{**}$	$F_{2,30}$=1.8	$F_{2,189}$=0.09	$F_{2,424}$=0.5
Food	$\mathbf{F_{2,38}=20.8}^{***}$	$\mathbf{F_{2,19}=9.5}^{**}$	$\mathbf{F_{2,191}=43.4}^{***}$	$F_{2,424}$=0.1
Density×food	$F_{4,1103}$=1.2	$F_{4,350}$=0.8	$F_{4,191}$=2.87	$F_{4,318}$=0.3
Sex	-	$\mathbf{F_{1,558}=39.5}^{***}$	$\mathbf{F_{1,190}=150.9}^{***}$	$F_{1,323}$=0.7
Sex×density	-	$F_{2,546}$=0.5	$F_{2,184}$=1.42	$F_{2,340}$=0.1
Sex×food	-	$F_{2.525}$=0.4	$F_{2,184}$=0.04	$F_{1,169}$=0.3
RANDOM EFFECTS				
Family	$\mathbf{\sigma^2=0.49}^{**}$	$\mathbf{\sigma^2=0.04}^{**}$	$\mathbf{\sigma^2=0.009}^{**}$	σ^2=-0.005
Family×density	σ^2=0.04	σ^2=0.03	σ^2=0.002	σ^2=-0.003
Family×food	σ^2=0.03	σ^2=0	σ^2=0.000	σ^2=-0.008
Family×density×food	$\mathbf{\sigma^2=0.68}^{**}$	$\mathbf{\sigma^2=0.05}^{**}$	σ^2=0.000	σ^2=-0.0001
Residual	σ^2=0.87	σ^2=0.22	σ^2=0.047	σ^2=0.27

Survival probability, body length and tibia length varied significantly among the different families but there were no significant genotype×environment interactions. Broad sense heritabilities equaled 0.48, 0.24 and 0.32 respectively. There appeared to be family×food-quality×density three-way interactions (Table 1), which are, however, likely to reflect common environment effects.

Individual unsigned asymmetry appeared to have no genetic component and there was no indication of genotype×environment interactions. All variance components were slightly smaller than zero, which may be the result of sampling variation (LYNCH & WALSH, 1997) but could also stem from negative within-family correlations (NELDER, 1954; THOMPSON, 1962; COCKERHAM, 1973; FALCONER & MACKAY, 1996). The broad sense heritability of FA equaled -0.036. Since this heritability is negative, a transformation applying the hypothetical repeatability is meaningless.

Individual unsigned asymmetry was weakly, but significantly positively correlated with individual body length (r_s=0.17, N=430, p=0.0002). Therefore, patterns in asymmetry to some extent might reflect patterns in body length. After removing the effect body length (a measure of individual quality, see above) by dividing individual asymmetry by body length, tibia length was no longer correlated with individual asymmetry (r_s=-0.01, N=430, p=0.8). Following Leung (1998) (i.e. rule 9) we divided individual asymmetry by tibia length to obtain a size-corrected asymmetry estimate. Re-analyzing this new asymmetry-parameter did not change the overall results.

Power considerations

Heritability of FA and DI

In order to generate data under a range of positive values of h^2_{DI}, we sampled data for 20 families with sample

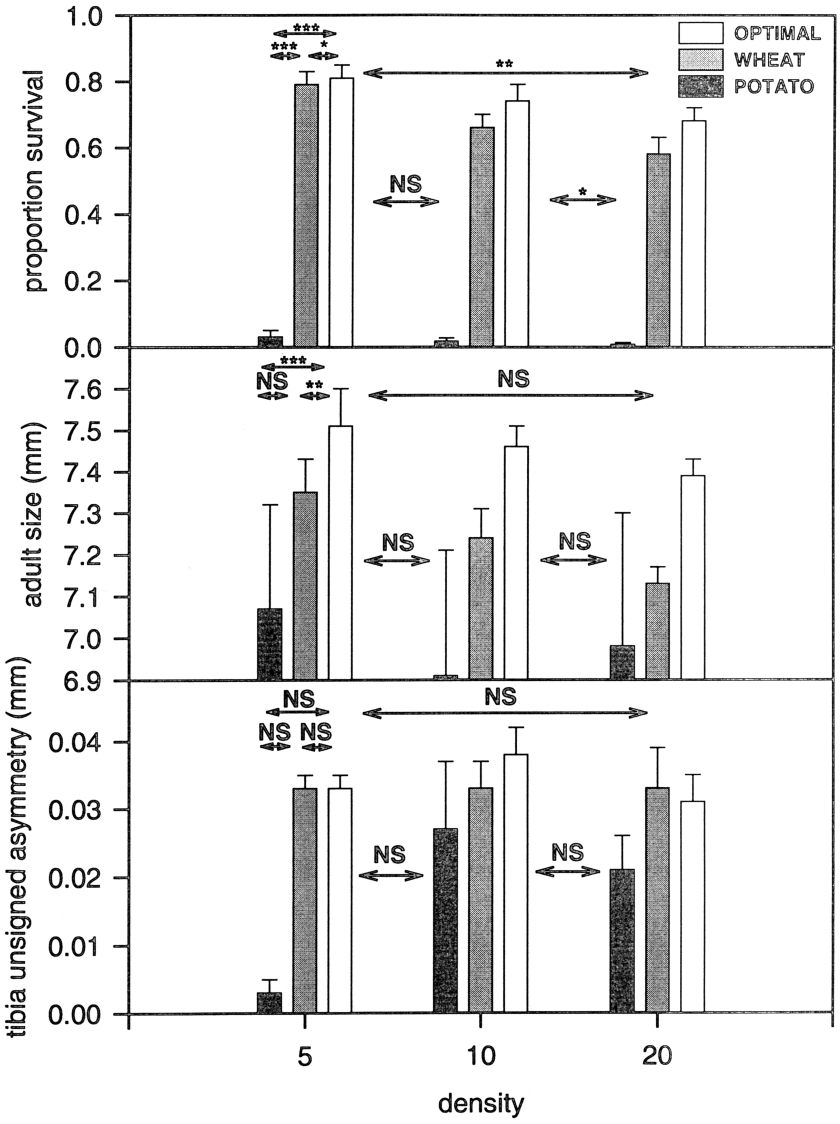

Fig. 1. – Survival probability, mean body length and mean individual unsigned asymmetry for different food qualities and densities (error bars indicate the standard error of the mean). Statistical significance of the different effects is indicated above the arrows (NS: $p>0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). Arrows between the different food qualities indicate effects averaged over the three densities since there was no food×density interaction (Table 1).

sizes, levels of DI, and heterogeneity in DI (i.e. hypothetical repeatability) equal to the observed values. Between-individual heterogeneity in DI was partitioned within and between families at different proportions, reflecting different heritabilities. For each value of h^2_{DI}, 10000 samples were generated, and the proportion of samples where the h^2_{FA}£-0.036 (i.e. observed h^2_{FA}) was used as the significance level, testing if the simulated h^2_{DI} was larger than the observed heritability just by chance alone. *P*-values were smaller than 0.05 for all $h^2_{DI}\geq 0$ (Fig. 2), indicating that h^2_{DI} is significantly negative. Obviously, a negative heritability does not indicate negative additive genetic variance. By definition, variances cannot be negative, however, their estimates can. It is usually assumed that negative variance components are the result of sampling variance (Lynch & Walsh, 1997), but negative within-family correlations may result in negative estimates as well (Nelder, 1954; Thompson, 1962; Cockerham, 1973; Falconer & Mackay, 1996). The significantly negative between-family variance in individual asymmetry found here suggests that asymmetry values are negatively correlated within families and not the result of statistical noise (i.e. random sampling variation).

Environmental effects on DI

Food quality had a strong effect on survival probability (Table 1, Fig. 1). If any, the strongest effect of environmental stress on FA would be expected at this level. However, only 14 moths survived in the POTATO treat-

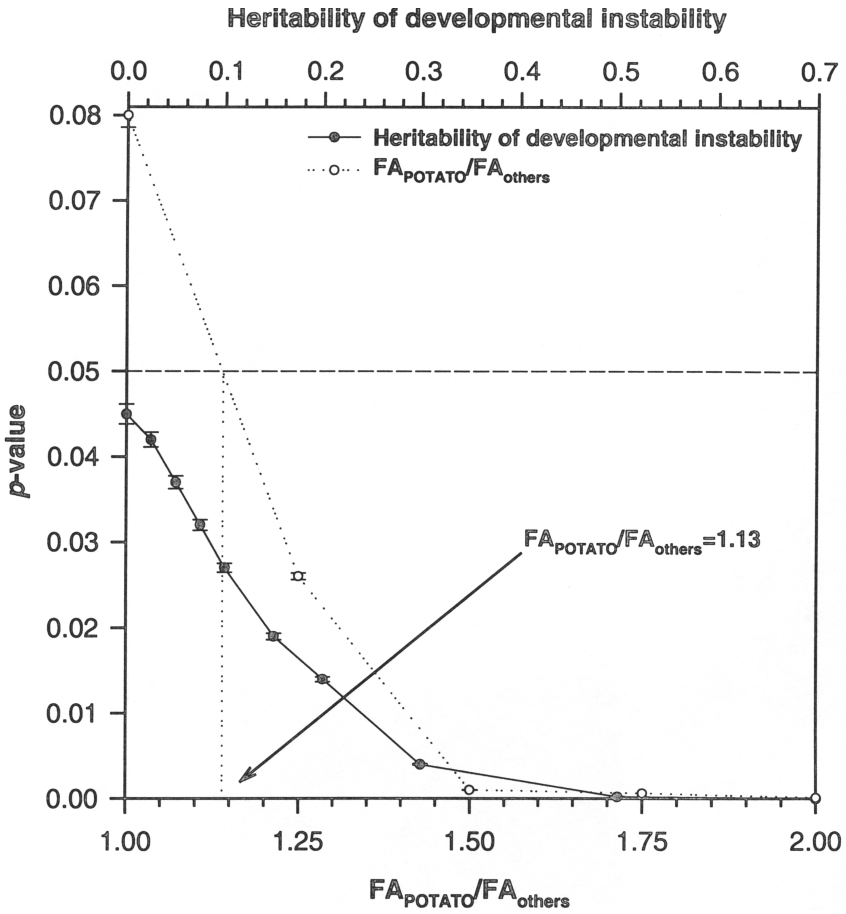

Fig. 2. – *P*-values of different alternative hypotheses testing for different values of h^2_{DI} (solid line and filled symbols) and for different ratios of population level unsigned asymmetry differences (i.e. moths reared on POTATO vs. all others) (dotted line and open symbols). Simulations of size 10,000 were performed to generate the empirical distributions (i.e. $h^2_{DI}>0$ and increased developmental instability on the food source with the lowest survival probability: $FA_{POTATO}/FA_{others}>1$). 95% CIs of the *p*-values were obtained following CRAWLEY (1993). The ratio corresponding to a *p*-value of 0.05 is indicated.

ment and statistical power to detect an effect on FA is small. Average individual unsigned asymmetry equaled 0.020 (N=14) for moths reared on POTATO compared to 0.033 (N=416) for all others. Thus, the difference in FA, although not statistically significant, is opposite to the expectations. We generated data for different degrees of heterogeneity in FA where FA for the POTATO reared moths was higher compared to the others ($FA_{POTATO}/FA_{others}\geq1$, i.e. heterogeneity in FA in the expected direction). For each level of heterogeneity we generated 10,000 samples of two populations with sizes 14 and 416 and used the proportion of samples where FA_{POTATO}/FA_{others} was smaller than 0.61 (i.e. 0.02/0.033) as p-value. In each simulation we incorporated a hypothetical repeatability of 0.2 (see above) because it has been shown to influence statistical power (VAN DONGEN, 1999b). This analysis indicates that FA_{POTATO} is maximally 13% larger than FA_{others} (Fig. 2) since larger differences would make the observed outcome very unlikely to have occurred by chance. Heterogeneity is expected to be even lower for the other comparisons since their effects on survival and body length were much weaker and statistical power much higher.

DISCUSSION

In this study we show that individual asymmetry of the front tibia does not provide a reliable estimate of individual quality in the Indian meal moth. In spite of significant between individual heterogeneity in DI (i.e. positive hypothetical repeatability), the heritability of DI was negative. This cannot be attributed to the fact that we worked with a culture reared in the laboratory since all other traits, including tibia length showed a significant broad sense heritable component. In addition, tibia asymmetry did not co-vary with fitness as manipulated by different nutritional qualities. Power analyses showed that possible undetected effects were likely to be very small. Because DI is not heritable in this population, we can rule out the hypothesis that under the presumed adverse food and den-

sity conditions only 'genetically superior' individuals with low DI would survive obscuring effects of stress. We therefore conclude that, in contrast to body length and survival probability, there is no evolutionary potential of tibia DI in this population of the Indian meal moth.

Heritabilities from full-sib breeding experiments are confounded with non-additive effects. They should be considered as upper bounds of narrow sense heritability only, and therefore cannot explain the negative heritabilities of FA and DI. Dominance effects may play an important role in both DI (LEAMY et al., 1997; 1998) and fitness traits (e.g. GARCÍA *et al.*, 1994) and may inflate broad sense heritabilities by over 30% (CRNOKRAK & ROFF, 1995). The main advantage of performing a full sib breeding design, especially considering the small heritabilities typically found for FA, is its increased power. Yet, maternal effects may obscure patterns of interest. Thus some caution is needed in interpreting the obtained results.

Our analyses show that tibia FA is not influenced by genetic or nutritional factors. Nevertheless, the hypothetical repeatability was significantly larger than zero. Hence, DI varied between individuals independent of genotype and food quality. In crowded situations, larvae of the Indian meal moth interact socially, emit secretions from their mandibular glands, and may show aggressive interactions and even become cannibalistic (GAGE, 1995 ; ANDERSON & LÖFQVIST, 1996). If in any of these interactions some individuals become dominant over others and if these interactions influence DI, it may be the source of the between-individual variation in DI observed. Possibly, small random initial differences in developmental rate may induce dominance of some individuals over others and in that way may lead to variation in DI at the individual level. This could also explain why h^2_{FA} was negative. If in each or many experimental treatments dominance of some individuals over others occurs and induces variation in DI, within-treatment (and also within-family) variation in individual asymmetry is increased, whereas the between family component remains unchanged or may even decrease. This would result in a negative correlation between asymmetries of individuals within jars and families and thus a negative value of h^2_{FA} (NELDER, 1954; THOMPSON, 1962; FALCONER & MACKAY, 1996) as observed in this study. We therefore could conclude that levels in DI in the front tibia of Indian meal moths may appear to be determined by social interactions irrespective of genotype and nutritional conditions.

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