

Fitness-heterozygosity associations differ between male and female winter moths *Operophtera brumata* L.

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ABSTRACT. The association between heterozygosity and fitness is positive but weak on average and varies between studies. Inbreeding has been invoked as the driving force between the positive heterozygosity-fitness associations, yet in spatio-temporally stable environments a negative correlation is expected. Furthermore, different patterns can arise because of the effects of natural selection on different loci and variation can be expected among groups of individuals that experience different levels of stress. In this paper we report on fitness-heterozygosity associations in the winter moth for six allozyme loci. The relationship is estimated for males and females separately, in four areas differing in their degree of fragmentation, and variation among loci is modelled. We introduce a linear mixed model framework to achieve this analysis. This approach differs from more traditional (multiple) regression analyses and allows testing specific interactions. We show that fitness, as estimated by body size, is negatively correlated with heterozygosity, but only so in females. This association does not vary significantly among loci and the four areas. We speculate that a trade-off between fitness-consequences of inbreeding and outbreeding at different stages of the winter moth life cycle could explain the observed patterns.

KEY WORDS : fitness, heterozygosity, inbreeding, outbreeding, natural selection, genetic drift, winter moth, Operophtera

INTRODUCTION

The extent to which the environment interacts with the genotype affecting fitness can influence population dynamics and even extinction risks of small endangered populations. Yet, this issue remains poorly investigated and often ignored in ecology and evolutionary biology (COULSON et al., 1998a). One aspect of the association between genotype and fitness that has received relatively much attention in the literature are correlations with degree of heterozygosity or inbreeding. Yet, the predicted positive association between genetic diversity and fitness is far from general (BRITTEN, 1996; MITTON, 1997), and little is known about the factors that affect this association (but see e.g., PALMER, 1996; MITTON, 1997; LESBARRÉRES et al., 2005). Recently, LESBARRÉRES et al. (2005) found heterogeneity in fitness-heterozygosity associations among geographic areas and SACCHERI et al (2005) found indications for differences between the two sexes.

How an association between heterozygosity and fitness can become established is a subject of many debates. Observed individual heterozygosity may reflect genome-wide heterozygosity and thus the overall levels of inbreeding and may reduce fitness (CHARLESWORTH & CHARLESWORTH, 1987). Yet, a large number of loci should be scored to obtain a reliable estimate of genome-wide heterozygosity drastically reducing power to detect a fitness-heterozygosity association. Failing to do so drastically reduces the power to detect an association. Since many studies do find an association with few loci, rather natural selection phenomena on single loci, or effects at tightly linked areas on the chromosome, are often thought

to be responsible for the observed fitness-heterozygosity association (MITTON, 1997). It is, however, unlikely that a single mechanism explains such a widespread phenomenon. Indeed, some studies using presumed selectively neutral microsatellite markers also found positive fitness-heterozygosity associations (COLTMAN et al., 1998; COULSON et al., 1998b; LESBARRÉRES et al., 2005) favouring the hypothesis of genome-wide effects of inbreeding over the selection theory. On the other hand, some detailed studies of the kinetics of specific metabolic enzymes have shown clear effects of selection on single locus genotypes (see MITTON, 1997 for references and details). HALDANE (1954) proposed that heterozygotes at enzyme loci would be more efficient than homozygotes at controlling flux in metabolic pathways. Under this hypothesis it is assumed that different genotypes have different properties under different environmental conditions and that heterozygotes would be superior when conditions fluctuate between those favouring the different homozygotes (MITTON, 1997). When environmental differences are persistent in space, however, genetic differentiation (either through the effects of genetic drift or natural selection) between the environments is expected to evolve, and mating between genetically distant individuals becomes disadvantageous as it disrupts local adaptation (i.e. outbreeding depression, e.g. STRAUSS & KARBAN, 1994; COULSON et al., 1998b). Therefore, in spatio-temporally stable selective environments, a negative association between fitness and heterozygosity is expected. In theory it should be possible to differentiate effects due to drift and selection. Genetic drift is expected to affect different loci in a similar way whereas the effects of natural

selection are more likely to differ among loci. It is therefore important to compare fitness-heterozygosity associations among loci.

Environmental stress may increase the expression of heterozygote advantage although the underlying mechanisms are only poorly understood (PALMER, 1996; MITTON, 1997). Fitness-heterozygosity associations may therefore differ among areas and/or groups of individuals that experience different levels of stress. Here we report on fitness-heterozygosity associations in the winter moth *Operophtera brumata* L. at 6 allozyme loci. The winter moth inhabits oak forests that differ in quality (degree of isolation and surface area) and levels of stress differ between males and females as they differ in their respiration levels and degree of weight loss during pupation. Males lose much more weight compared to females and suffer higher mortality during pupation (GRADWELL, 1974). We study four areas that differ with respect to area and degree of isolation (see below for description). Earlier results confirmed that these factors influence different aspects of the population structure and fitness in particular (VAN DONGEN et al., 1994; 1998a; VAN DONGEN, 1997). Considering the importance of comparing associations among loci, areas and sex we introduce a statistical framework based on linear mixed models to perform these analyses with maximal power.

MATERIALS AND METHODS

Study species

Winter moth egg hatching is to some extent locally synchronised to the individual-specific budburst dates of pedunculate oak *Quercus robur* L. (VAN DONGEN et al., 1997). Within single study plots, neighbouring trees consistently differ by up to 4 weeks in budburst date creating a highly heterogeneous environment on a very small geographical scale (CRAWLEY & AKHTERUZZAMAN, 1988; VAN DONGEN et al., 1997). This local adaptation is the result of the strong negative fitness effects on caterpillars hatching out of synchrony (FEENY, 1968; 1970; GRADWELL, 1974; WINT, 1983; VAN DONGEN et al., 1997). Because egg-hatching date has a genetic basis (SPEYER, 1938; 1941) dispersal of locally adapted individuals is likely to be disadvantageous and outbreeding depression and negative fitness-heterozygosity associations are expected to occur in this species. The winter moth is a particularly interesting species to study the fitness-heterozygosity associations because males and females are subject to different levels of stress during pupation. Males consume much more oxygen, lose more weight and suffer higher mortality during pupation than females (GRADWELL, 1974). If stress has an effect on the fitness-heterozygosity association, males and females will show a different association. Furthermore, oak forests have become highly fragmented in N-Belgium, as in other parts of the world. Fragmentation has been shown to affect winter moth fitness/weight in our study area (VAN DONGEN et al., 1994; 1998a).

Sampling and electrophoresis

Male and female winter moths were collected in copula on individual trees (N = 520 couples) at night by hand during November 1994 in four different areas near Antwerp, Belgium. All study sites were dominated by pedunculate oak that showed up to four weeks of consistent variation in budburst date. Still, areas differed considerably with respect to area, isolation and structure: area1 (51°16' N, 4°30' E): part of a forest-parkland complex of well over 200 ha on the suburban fringe of Antwerp (N = 105 couples); area2 (51°07' N, 4°32' E): a small oak forest fragment of 1.6ha with a low degree of isolation (N = 334 couples); area3 (51°09' N, 4°32' E): a small oak forest fragment of 0.5ha with a relative high degree of isolation (N = 41 couples); and area4 (51°08' N, 4°30' E): an oak lane of 400m length (N = 40 couples). After collection, moths were stored at -80°C for electrophoretic analysis. A total of six enzyme loci with relative high degree of genetic variation were scored [peptidase (PEP: E.C. 3.4.11.*, with leucylalanine as substrate), phosphoglucosaminase (PGM: E.C. 5.4.2.2), glucose-phosphate isomerase (GPI: E.C. 5.3.1.9), 6-phosphogluconate dehydrogenase (PGD: E.C. 1.1.1.44, detectable in males only), b-hydroxybutyrate dehydrogenase (HBDH: E.C. 1.1.1.30), alkaline phosphatase (ALP: E.C. 3.1.3.1, detectable in females only) with vertical polyacrylamide gel electrophoresis (details in VAN DONGEN et al., 1994; 1998a). Bodyweight after log-transformation (to obtain approximate normality) was used as an estimate of individual fitness. Weight is closely correlated to other measures of body size and reflects several components of fitness such as female fecundity, male and female mating success, adult lifespan, and pupal survival (FEENY, 1970; GRADWELL, 1974; WINT, 1983; VAN DONGEN et al., 1997; 1998b; 1999). Body weight could also be obtained most reliably after moths were frozen and defrosted again. We also wanted to avoid as much time as possible during the preparations of the samples for electrophoresis to assure that gels could be reliably scored.

Statistical analysis

To analyse the association between weight and heterozygosity a mixed model approach (LAIRD & WARE, 1982) was performed in SAS (ver. 6.12) used to evaluate variation among loci, areas and gender. In this analysis, each observed individual weight (logtransformed) was used five times, corresponding to the five different loci for each sex. In this way, the factor locus and relevant interactions could be added to the model as random effects. This approach differs from traditional regression analyses. The advantage is that it allows addressing particular hypotheses directly by testing specific interactions, rather than making inference indirectly from presence or absence of associations (e.g. LEARY, KNUDSEN & ALLENDORF, 1983). Because each individual weight is used five times, residual values are correlated within individuals. To assure that the degrees of freedom and consequently the type-I error rate were not inflated due to this statistical dependency, the correlation between these residual values was modelled explicitly and degrees of freedom were

approximated by Satterthwaite's procedure (LITTELL et al., 1996; VERBEKE & MOLENBERGHS, 1997). This was achieved by adding the factor individual as a random effect to the model (LITTELL et al., 1996; VERBEKE & MOLENBERGHS, 1997). The Satterthwaite procedure makes use of this correlation to adjust the degrees of freedom and to assure that the Type-I error rate equals the nominal level of 5%. In case there was no indication of any locus-effect the analysis was continued by relating individual weight to heterozygosity averaged over the five loci. In this way, each individual was used only once in the analysis so that the data were statistically independent. For some individuals, some loci could not be scored unambiguously. To avoid potential bias, only individuals for which all genotypes were known ($N = 571$) were included. Nevertheless, analyses including all available data showed similar patterns (data not shown).

Next to the random locus effect, area and relevant interactions were added as random effect, in order to model how the weight-heterozygosity relationship varied among the four study areas. As fixed effects, heterozygosity, sex and their two-way interaction were included. Fixed effects were tested by F-tests while significances of the random effects were obtained from

likelihood ratio tests (as outlined in VERBEKE & MOLENBERGHS, 1997).

RESULTS

There appeared to be no detectable variation in the weight-heterozygosity association among the six loci because all variance component estimates equalled zero (Table 1). Therefore, the analysis was continued with the average individual heterozygosity. Neither the weight-heterozygosity relationship nor adult weight varied among the four study areas as judged from the non-significant area related random effects (Table 1). There was a significant relationship between weight and heterozygosity, and this relationship differed between males and females (Table 1): weight decreased significantly with heterozygosity in females but not in males (Fig. 1). The residuals of the finally selected model were approximately normally distributed. We also examined whether some alleles were specifically associated with weight variation, yet after correction for multiple testing, none of these tests were statistically significant. However, due to the high number of comparisons, statistical power was probably low.

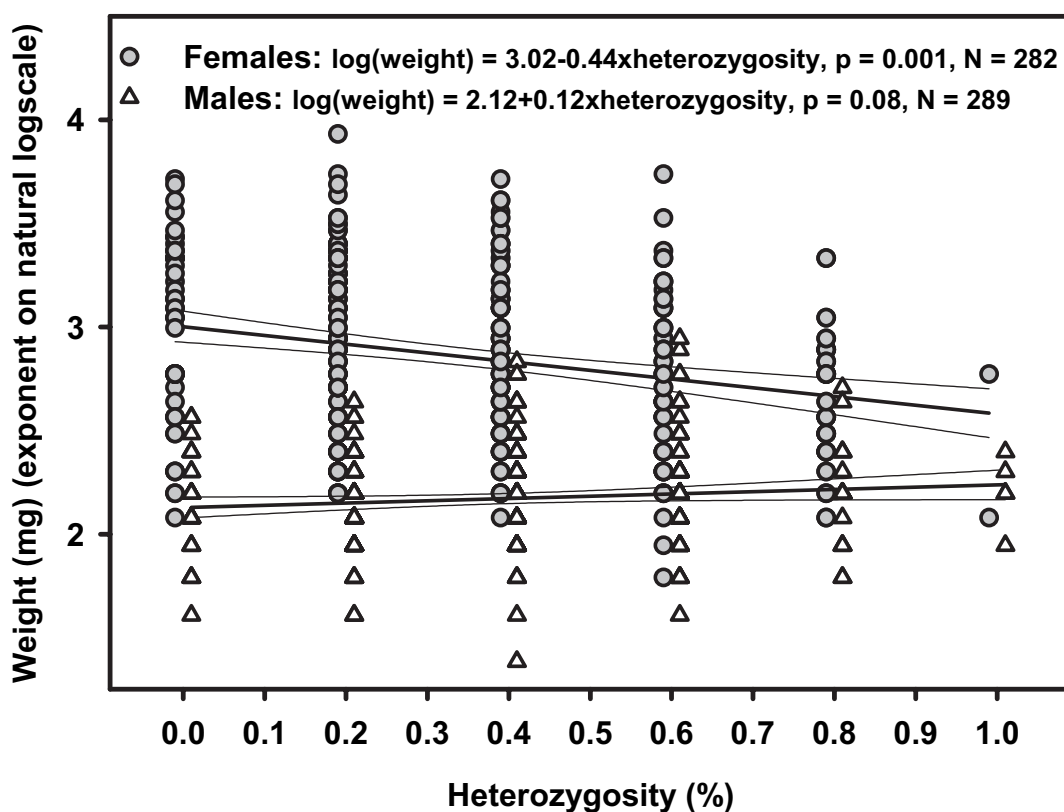


Fig. 1. – Relationship between individual heterozygosity as estimated from five polymorphic enzyme loci and individual weight (on a logscale) for males and females. Data from different areas and loci were pooled because the relationship did not differ between the different levels of these factors (see results and Table 1). Regression lines and their 95% confidence intervals are indicated for both sexes.

TABLE 1

Summary of the fixed and random effects in the mixed model relating male and female body-weight to heterozygosity at six allozyme loci in four areas.

Fixed effects	F-test statistic ^a	d.f. 1	d.f. 2	p-value
Sex	199.0	1	568	0.0001
Heterozygosity	5.0	1	568	0.03
Sex × heterozygosity	15.6	1	568	0.0001
Random effects	σ^2	χ^2 test statistic ^b	d.f.	p-value
Area	0.003	0.2	1	0.95
Area × sex	0	–	–	>0.05
Area × heterozygosity	0.001	0.1	1	0.97
Area × sex × heterozygosity	0	–	–	>0.05
Locus	0	–	–	>0.05
Locus × sex	0	–	–	>0.05
Locus × heterozygosity	0	–	–	>0.05
Locus × sex × heterozygosity	0	–	–	>0.05

a. Fixed effects were tested by means of traditional F-tests with d.f. approximated by Satterthwaites procedure.

b. Random effects were tested by likelihood ratio test when the estimated variance component was larger than zero.

DISCUSSION

In contrast to many other studies we find a negative relationship between fitness and heterozygosity, a correlation that appeared constant across loci and areas, but only present in females. This negative association suggests the presence of outbreeding depression. Local adaptation of winter moth egg hatching to host tree budburst may have resulted in a genetic differentiation of the loci under investigation, i.e., a balanced polymorphism. Mating between individuals that are adapted to trees with different budburst phenology will then result in more asynchronous offspring with lower fitness and higher heterozygosity. Unfortunately, this presumed genetic differentiation could not be quantified accurately because of low number of moths collected on individual trees. Yet, deviations from Hardy-Weinberg equilibrium (heterozygote deficit) at the level of the study site, as reported earlier by VAN DONGEN (1997), suggest the presence of such a genetic substructuring.

We can only speculate about the reasons for the observed differences in males and females. Adult size is determined by two factors: the amount of food a caterpillar accumulates and thus its degree of synchrony with the individual host (VAN DONGEN, 1997), and the weight loss during pupation (GRADWELL, 1974). We argued that heterozygosity could be associated with larval synchrony (VAN DONGEN, 1997), an association that is unlikely to differ between males and females during the caterpillar stage. Thus, the significant difference in the weight-heterozygosity association between males and females is likely to result from effects occurring during pupation. Males suffer higher mortality, more weight loss and consume more oxygen during pupation than females (GRADWELL, 1974).

Because heterozygous individuals have been shown to have a higher metabolic efficiency in several species (MITTON, 1997), it is possible that heterozygous males, although lighter at the start of pupation due to lower synchrony, lose less weight during pupation, reversing or counteracting the earlier established negative weight-heterozygosity association. In addition, the higher heterozygosity in males than in females (VAN DONGEN, 1997) is in agreement with this hypothesis. To test this hypothetical explanation formally, it is necessary to investigate weight-heterozygosity associations in caterpillars, to monitor weight loss during pupation for individuals differing in their degree of heterozygosity and/or to compare the observed associations with those for a selectively neutral marker (e.g. microsatellite loci).

The observed associations between fitness and heterozygosity can potentially affect several aspects of the population structure. The presumed higher mortality in less heterozygous males not only affects the sex ratio and effective population size, but may also limit the evolution of local adaptation of egg hatching to host budburst phenology. The synchrony between the latter two is statistically significant, yet relatively weak ($r^2 < 32\%$, VAN DONGEN et al., 1997) or even absent in some years or areas (VAN DONGEN, unpublished results). This could be explained by the lower fitness-benefits of synchrony for males as it results in lower heterozygosity and consequently higher pupal mortality.

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