



Inbreeding depression and the maintenance of genetic load in *Melitaea cinxia* metapopulations

Sari Haikola^{1*}, Wilhelm Fortelius², Robert B. O'Hara³, Mikko Kuussaari⁴, Niklas Wahlberg⁵, Ilik J. Saccheri⁶, Michael C. Singer⁷ & Ilkka Hanski³

¹Tvärminne Zoological Station, FIN-10900 Hanko, Finland; ²Sydväst Polytechnic, Forstintitutsvägen, FIN-10600 Ekenäs, Finland; ³Department of Ecology and Systematics, Division of Population Biology, P.O. Box 17 (Arkadiankatu 7), FIN-00014 University of Helsinki, Finland; ⁴Finnish Environment Institute, Nature and Land Use Division, P.O. Box 140, FIN-00251 Helsinki, Finland; ⁵Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden; ⁶Division of Population and Evolutionary Biology, School of Biological Sciences, University of Liverpool, Nicholson Building, Brownlow Street, Liverpool L69 3GS, UK; ⁷Section of Integrative Biology, University of Texas, Austin, Texas 78712, USA (*Author for correspondence: sari.haikola@helsinki.fi)

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Abstract

The effects of inbreeding on fitness and the maintenance of genetic load in metapopulations of the endangered Glanville fritillary butterfly (*Melitaea cinxia*) were examined in four laboratory experiments. In Finland *M. cinxia* occurs as a large metapopulation consisting of small local populations with fast turnover, whereas in southern France the species has a more continuous population structure. In the experiments, we compared the performance of crosses between full sibs, crosses between members of different families within populations, and crosses between individuals from different populations. These experiments were replicated using insects from two different regions, Finland and southern France, between which the frequency of natural inbreeding should differ substantially because of differing population structure. In Finnish butterflies, the rate of successful mating was lower among insects derived from small than from large natural populations, probably reflecting the effect of past inbreeding history. Mating between full sibs lowered egg hatching rate in all experiments. This reduction of egg hatching rate was more severe among French butterflies with a more continuous population structure than among Finnish butterflies with small naturally fragmented populations and with a history of repeated rounds of inbreeding in the past. This result suggests that recurrent inbreeding has led to partial purging of deleterious recessives from the Finnish metapopulation. Nonetheless, substantial genetic load still remains in this metapopulation, and we discuss possible reasons why this should be the case.

Introduction

Inbreeding generally lowers the viability and fertility of individuals (Falconer and Mackay 1996, pp. 247–262; Hedrick and Kalinowski 2000; Ralls and Ballou 1983), and inbreeding can have specific negative effects on growth (e.g. Stevens and Bougourd 1988), survival (e.g. Jimenez et al. 1994), and many other fitness components (see Frankel and Soule 1981, pp. 59–77 for a review). The consequences of

inbreeding are likely to be often underestimated due to difficulties of measuring inbreeding depression in all life stages (Charlesworth and Charlesworth 1987). It has often been suggested that inbreeding depression should be low in typically inbred populations due to purging (Lacy and Ballou 1998; see also Bataillon and Kirkpatrick 2000 for a theoretical examination of the effects of population size on inbreeding depression and genetic load), but there is also evidence suggesting that even highly inbred populations can suffer from

substantial inbreeding depression (Charlesworth and Charlesworth 1987; Hedrick and Kalinowski 2000; see also Byers and Waller 1999 for a review of studies on purging inbreeding depression; Kirkpatrick and Jarne 2000 for a theoretical examination of inbreeding depression and genetic load following a population bottleneck).

The experiments reported here describe the effects of inbreeding on viability of the Glanville fritillary butterfly (*Melitaea cinxia*) and the maintenance of genetic load in a metapopulation of this species in the Åland Islands in SW Finland. *Melitaea cinxia* went extinct on mainland Finland in the 1970s, and is now restricted to the Åland Islands in Finland (Marttila et al. 1990), where it occurs in a large network of discrete habitat patches (dry meadows), persisting in a balance between local extinctions and recolonizations (Hanski et al. 1995a; Hanski 1999, pp. 207–232). Local populations are typically very small and relatively isolated, often consisting of just one larval family, in which case all individuals in the population will be siblings (Hanski et al. 1995a; Hanski 1999, pp. 207–232). Due to the highly fragmented population structure, brother-sister matings must occur regularly in natural populations of *M. cinxia* in the Åland Islands.

In the years 1993–1996, some 1,600 meadows suitable for *M. cinxia* in Åland were monitored. Population turnover rate was high, with an average of 200 extinctions and 114 colonizations observed per year (Saccheri et al. 1998). Many factors influence the risk of local extinction, including population size (demographic stochasticity), environmental stochasticity, the sizes of the neighbouring populations (the rescue effect), as well as various characteristics of the habitat patches (Hanski et al. 1995b; Hanski 1999, pp. 207–232). Of particular interest in this context is that, after correcting for the influence of the above-mentioned ecological factors, the extinction risk of local populations increased significantly with decreasing heterozygosity, which was interpreted as an effect of inbreeding (Saccheri et al. 1998). A recent study by Nieminen et al. (2001) has produced more direct experimental evidence that inbreeding increases the extinction risk in small local populations of *M. cinxia* in Åland.

In contrast to the situation in Åland in Finland, *M. cinxia* is widespread in many parts of southern Europe. In southern France, *M. cinxia* appears to be present where habitat is available much more predictably than in Åland. Rough figures for occupancy of

suitable meadows are 80% in France (M. Singer and I. Pen, personal observations) and 20% in Åland (Hanski 1999, pp. 207–232). The much higher level of habitat occupancy in France most likely reflects the more continuous population structure there than in Åland.

In this study, laboratory experiments were used to compare butterflies originating from the fragmented populations in Åland with butterflies originating from the more continuous populations in southern France. The experiments were conducted by crossing individuals with known family and recent population history. Unfortunately, the rearing of *M. cinxia* in captivity poses several problems, which have prevented more elaborate experiments. Nonetheless, given the interesting ecological setting of the experimental populations, the results of even limited laboratory experiments make a valuable contribution. In particular, it is especially important to document the level of inbreeding depression on fitness components for the Åland populations, because our previous observational (Saccheri et al. 1998) and experimental studies (Nieminen et al. 2001) have suggested that inbreeding significantly increases the risk of population extinction in these populations. Furthermore, the comparison with the more continuously distributed, and presumably less inbred, French populations provides empirical data on the influence of metapopulation structure on genetic load. The expectation was that inbreeding depression would be less severe in the Finnish metapopulation due to partial purging (Wang 2000).

Material and methods

Laboratory experiments

Laboratory experiments on checkerspot butterflies such as *M. cinxia* are often complicated by low rates of successful matings and high mortality among diapausing larvae. For these reasons we have been unable to continue rearings for multiple generations. Instead, we have conducted several independent experiments to examine the possible effects of brother-sister matings on mating success, egg hatching rate and larval survival. Such experiments are relevant for the ecological situation in the large metapopulation in the Åland Islands, where many populations are short-lived and consist of closely related individuals.

Mating success

Experiment 1 (spring 1995). The aim of this experiment was to study the effects of inbreeding on mating success. Larvae for this experiment were collected from four small populations and four large populations (from several families in each large population) in the Åland Islands in spring 1994. The small populations consisted of only 1–2 larval families at the time of sampling and were relatively isolated from other populations, whereas the large populations had 9–22 larval families and were well-connected (Figure 1; for a description of the metapopulation structure of *M. cinxia* in the Åland Islands see Hanski et al. 1995a; Hanski 1999, pp. 207–232). The larvae were reared to butterflies, which were allowed to mate randomly with individuals from the same population. The offspring were reared to butterflies, and this second generation was used in the mating experiment. The butterflies from the small populations had thus experienced substantial inbreeding for at least two generations, whereas the level of inbreeding in the butterflies from large populations can be assumed to be very low.

Matings took place in indoor cages (length 40 cm, diameter 15 cm). There were 19 mating cages with butterflies from small populations and 23 cages with butterflies from large populations. Each mating cage had 2 to 22 butterflies. There were butterflies from each of the eight populations in at least four different cages. Dead butterflies were collected three times a week. Female butterflies were frozen at -20°C for scoring the presence/absence of spermatophores. The frequency of mated females was recorded on the basis of the presence of a spermatophore and the observed matings (females were not dissected if they had been observed to mate for the usual length of time).

Egg hatching rate

Experiment 2 (summer 1997). The aim of this experiment was to study the effects of inbreeding on egg hatching rate. Butterflies used in this experiment represented the third generation of butterflies from four distant areas in the Åland Islands (Figure 1) and were in some cases the product of brother-sister mating. Three types of crosses were made: (1) crosses within families, (2) crosses between members of different families within populations, and (3) crosses between individuals from different populations. Matings took place in large outdoor cages (diameter 41 cm, height 47 cm).

In the crosses within families, there were 74 females representing the four distinct areas in Åland. In the crosses between families and between populations, 55 females were successfully mated, representing eight different combinations of the four areas. Each individual mated only once. Mated females were placed in small cages with plastic cups containing cut leaves of *P. lanceolata* or *V. spicata*, on which females laid their eggs. When an individual female started laying, she was removed together with the cup from the cage and allowed to complete laying in isolation. Egg hatching rate was defined as the proportion of hatched eggs from those laid. The egg hatching rate was measured by collecting egg batches of individual females, spreading the eggs out on a Petri dish, taking a photocopy of the dish, and counting the eggs from an enlarged photocopy. Following an incubation period at $19\text{--}26^{\circ}\text{C}$ and 70–90% relative humidity, newly-hatched larvae were counted from a second photocopy.

There was no systematic difference in incubation temperature between the treatments as the larvae hatched during the same time period in all treatments. We do not know to what extent the method of counting eggs affects their survival, but there is no reason to suspect that eggs in the different treatments would be differently affected. Counting eggs without touching the egg batch is impossible because they are laid in a mass on top of each other.

Experiment 3 (summer 1998). The aim of this experiment was to compare the effects of inbreeding on egg hatching rate in populations representing different metapopulation structures. The material for this experiment was collected from seven distinct areas in the Åland Islands (Figure 1) and from eleven distinct populations in southern France in Provence and Languedoc-Roussillon.

The Åland material was collected as eggs. Hatched larvae were reared indoors until diapause, during which stage the larvae were kept in an outdoor shed. After diapause larvae were reared indoors to adult butterflies, which were used in the experiment. The French material originated from the eggs of wild-caught mated females. Hatched larvae were reared indoors without diapause to adult butterflies, which were used in the experiment (unlike in Åland, the diapause is facultative in southern France). The two sets of butterflies were studied concurrently to eliminate the possibility that the comparison would be affected by environmental conditions.

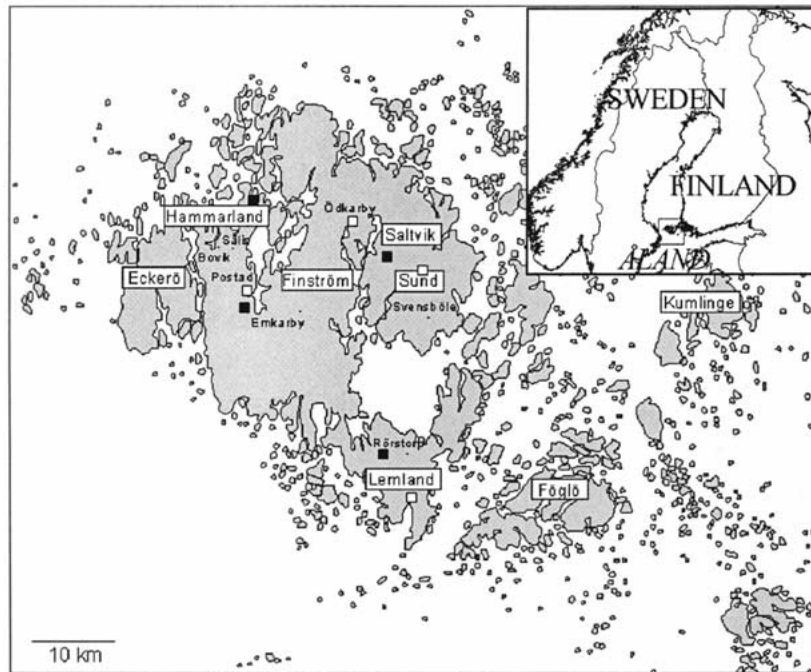


Figure 1. Locations of the study populations in the Åland Islands. Black squares refer to the small populations and white squares to the large populations in Experiment 1. In Experiment 2, the butterflies used in the within-family crosses originated from populations in Hammarland (Sålis and Postad), Kumlinge, and Föglö, and the butterflies in the crosses between families/populations from populations in the same areas. In Experiment 3, the butterflies used in the within-family crosses originated from populations in Hammarland (Postad and Bovik), Saltvik (Ödkarby), Lemland (Rörstorp), and Sund (Svensböle), and the butterflies in the crosses between populations from populations in Hammarland (Postad and Bovik), Saltvik (Ödkarby), Eckerö, and Finström (Emkarby).

Two types of crosses were used in this experiment: (1) crosses within families and (2) crosses between individuals from different populations. In the within-family crosses from Åland, there were 25 females from 15 families representing 5 distinct areas in the Åland Islands. In the between-population crosses we used 9 different combinations of 5 different areas, with a total of 68 females. The French within-family crosses included 11 populations, 21 families, and 82 individual females, whereas the crosses between populations consisted of 16 different combinations of 11 populations and included 52 females. Mated females were isolated and placed into little cages with a living plant for egg laying. Egg hatching rate was measured as in Experiment 2.

Post-diapause survival

Experiment 4 (summer 1997). As part of an observational study on inbreeding and population extinction (Saccheri et al. 1998), we studied the performance of larvae originating from small isolated versus

large well-connected populations in field and laboratory conditions. Mothers of the larvae reared in this experiment were collected from different parts of Åland in 1996. Average heterozygosity of the mothers was examined by assaying polymorphism at seven enzyme loci (*Ak*, *Got-1*, *Idh-1*, *Pep A*, *Pep D*, *Pgi*, and *Pgm*) and a microsatellite locus (Saccheri et al. 1998). Taking into account the population structure of *M. cinxia* in Åland, it is reasonable to assume that average heterozygosity reflects different levels of inbreeding among the local populations (Saccheri et al. 1998).

Larval groups whose mothers' heterozygosity was known were placed on a large unoccupied meadow on an isolated island in southwest Finland to diapause, with the aim of exposing all the larval groups to the same environmental conditions. In April 1997, ca 20 larvae per family from 30 different families and 24 populations were collected and weighed. Larvae were reared to adults on potted *P. lanceolata* plants at 15–20 °C and 75–85% relative humidity. Larvae were weighed four more times every fifth day. Pupae were

collected one day after pupation. After weighing the pupae were placed in plastic boxes and kept at 17–22°C and 60–75% relative humidity. Adult butterflies were kept in large cages (diameter 41 cm, height 47 cm) at 15–21 °C and 65–90% relative humidity.

Statistical analyses

Mating success

The mating success was analyzed using a generalized linear model (Genstat 5, Release 3.2). The numbers of matings in each cage were assumed to follow binomial distribution with the number of females as the binomial total and using a logit link function. The explanatory variables were the number of males in the cage, the number of females, population size category (small versus large), replicate populations (4 + 4) within the population size categories, and interactions between these variables. The *P* values were calculated assuming that the deviance followed a χ^2 -distribution.

Egg hatching rate

A preliminary analysis of egg hatching rate in Experiments 2 and 3 within each data set was performed using a generalized linear model (Genstat 5, Release 3.2). The data consisted of several nested levels. The numbers of hatched larvae were assumed to be binomially distributed, with a logit link function and egg number as the binomial total. The data were overdispersed, and we estimated the dispersion factor from the data. The female level is the lowest level in the hierarchy, and so forms the residual term in the analyses. The residual effect was tested against the χ^2 -distribution. The higher level terms were tested against the *F*-distribution with the mean deviance of the next lower level term as the denominator. Females with no hatched eggs were not included in the analyses, because they may have remained unfertilized even if they had been observed to mate. The numbers of such females are reported separately. Different females produced different numbers of clutches (from 1 to 5), partly depending on the butterfly's life span after mating, and we pooled data for all clutches for single females. The final analysis with all the data sets (Experiments 2 and 3) combined was done using the same method. The explanatory variables in the final analysis were *experiment* (the material from Åland and France in Experiment 3 were regarded as different experiments), *treatment* (crosses within

families versus crosses between populations/families), *populations* within the experiments and the *interactions* between these variables. The experiment, treatment and experiment*treatment terms were tested against the (experiment*treatment)/population term. The same analysis was repeated for the material from Experiment 3 only.

We also analyzed clutch size at all possible levels of nesting within the data sets. The family level was not used and the sizes of the first clutches only were recorded in Experiment 2. Clutch size was analyzed with an Analysis of Variance (Genstat 5). The population, cross (combinations of populations) and population/family terms were tested against the m.s. of the next lower level term. Differences in clutch size between the experiments and the treatments and the interaction between these factors were analyzed in the combined data set for all experiments and treatments (the data sets from Åland and France in Experiment 3 were treated as different experiments). The relationship between clutch size and egg hatching rate was analyzed with generalized linear models with clutch size as the explanatory variable. The numbers of eggs that hatched (out of the entire clutch) were modelled as being binomially distributed, with a logit link function and clutch size as the binomial total. The data were overdispersed, hence we estimated the dispersion factor from the data. Clutches of females with no hatched eggs were not included in the analyses.

Results

Mating success

The population size category had a large effect on mating success (Table 1, Figure 2), with the numbers of matings being significantly (Table 1) and substantially smaller (Figure 2) amongst butterflies originating from small and isolated populations than amongst butterflies originating from large and well-connected populations. Somewhat surprisingly, larger numbers of butterflies in the cages resulted in reduced frequencies of successful matings, especially in the case of butterflies originating from large populations (where large numbers of matings were observed, Figure 2). This effect is possibly due to interference amongst butterflies. The effect of population identity on mating success was not significant (Table 1), indicating a consistent difference in the mating success between the two population type

Table 1. Analysis of factors affecting the proportion of mated females in Experiment 1. The P value was calculated on the assumption that the deviance follows the χ^2 -distribution. PopSize = small isolated versus large well-connected populations. Popul = population identity

Source	d.f.	Deviance	P
Male	1	8.609	0.003
Female	1	4.175	0.041
PopSize	1	20.770	<0.001
PopSize.Popul	6	5.893	0.435
Male.Female	1	0.985	0.321
Male.PopSize	1	0.220	0.639
Female.PopSize	1	1.080	0.299
Male.Female.PopSize	1	13.966	<0.001
Male.Popsize.Popul	6	8.517	0.203
Female.Popsize.Popul	5	4.748	0.447
Male.Female.PopSize.Popul	5	2.941	0.709
Residual	12	13.025	
Total	41	84.929	

categories. The overall frequency of matings was low, apparently because the experiment took place in the spring (March–April) with limited amount of day light. Nonetheless, both population types experienced identical unfavorable experimental conditions for mating.

Egg hatching rate

Experiment 2. The average egg hatching rates and clutch sizes for crosses within families and between families/populations are presented in Table 2. It is apparent that the egg hatching rate was lower in the within-family crosses in comparison with crosses between families and populations (Table 2, Figure 3). The difference in the mean egg hatching rate between the treatments is partly due to differences between females within treatments (Table 3).

There was no significant association between egg hatching rate and clutch size (clutches of females with no hatched eggs excluded). Different crosses between families/populations ($N = 8$) differed in clutch size (ANOVA, $P = 0.007$), but crosses within families did not show significant variation in clutch size. The former differences may be due to some crosses with only one female with a low number of eggs.

Experiment 3. The difference between the treatments in egg hatching rate is comparable to that in Experi-

ment 2, the egg hatching rate being substantially lower in the within-family than between-population crosses (Table 2). As in Experiment 2, the difference in the mean egg hatching rate between the two types of crosses in the material from Åland is partly due to differences between females within treatments (Table 4). Egg hatching rate was positively correlated with clutch size in the crosses between populations ($P < 0.001$) but not in the crosses within families (clutches of females with zero egg hatching rate excluded). In the latter case there were no differences in clutch size at any level, whereas in the crosses between populations clutch size differed between females ($P = 0.003$).

The results for the French populations are qualitatively similar to the results for the Åland populations (Table 4). However, in the French populations egg hatching rate was correlated with clutch size in the crosses within families ($P = 0.03$, not significant after correction for multiple comparisons), but not in the crosses between populations (clutches of females with zero egg hatching rate again excluded). In the latter crosses there were no significant differences in clutch size between different combinations of populations, but clutch size differed significantly between the females ($P = 0.001$). In the crosses within families there were significant differences between the families ($P = 0.03$).

In the combined data set for all experiments and treatments clutch size differed between the experiments (ANOVA, $P < 0.001$) but not between the treatments, and the interaction was not significant. The difference between the experiments was due to a substantially higher average clutch size in Experiment 2 than in Experiment 3 (Table 2).

In the combined data set of Experiments 2 and 3, the egg hatching rate differed significantly between the treatments ($P < 0.001$). A fair comparison between the Finnish and French populations includes the data set from Experiment 3 for the Finnish population, because this experiment was run concurrently with the experiment on the French population, and hence the environmental conditions were the same. In Experiment 3 inbreeding reduced average egg hatching rate from 72% in the crosses between populations to 66% in the crosses within families in the material from Åland, whereas in the French material the corresponding decrease was from 76% to 44%. This difference in inbreeding depression between the Finnish and French populations is statistically significant (the experiment*treatment interaction term; Table 5).

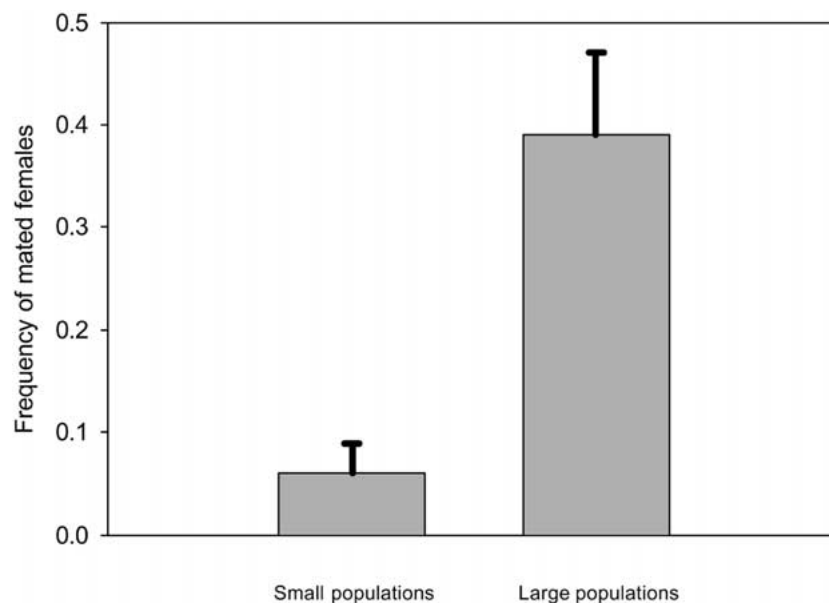


Figure 2. Frequency of mated females in Experiment 1, mean with SE in small (mean = 0.06, SE = 0.03, N = 19) and large (mean = 0.39, SE = 0.08, N = 23) populations. For statistical analysis see Table 1.

Table 2. Average egg hatching rate, clutch size and percentage of females with no hatched eggs (zero) in Experiments 2 and 3

Experiment	Within families						Between families/populations					
	Hatch rate		Clutch size			zero	Hatch rate		Clutch size			zero
	%	SD	mean	SD	n		%	SD	mean	SD	n	
Experiment 2	60	22.7	139	58	65	12	79	17.4	143	69	48	13
Experiment 3												
Åland	66	23.9	95	54	43	16	72	27.3	98	51	115	10
France	44	24.3	102	50	107	6	76	23.8	110	55	81	8
Pooled	53	25.4	112	56	215	10	75	24.6	111	59	244	10

Table 3. Analysis of egg hatching rate in Experiment 2. The residual (Population/Female) effect was tested against the χ^2 -distribution

Crosses within families				Crosses between families/populations			
Source	d.f.	Deviance	P	Source	d.f.	Deviance	P
Popul	3	28.24	0.80	Cross	7	261.44	0.12
Popul/Female	61	1705.50	0.00	Cross/Female	40	845.00	<0.001
Total	64	1733.74		Total	47	1106.44	

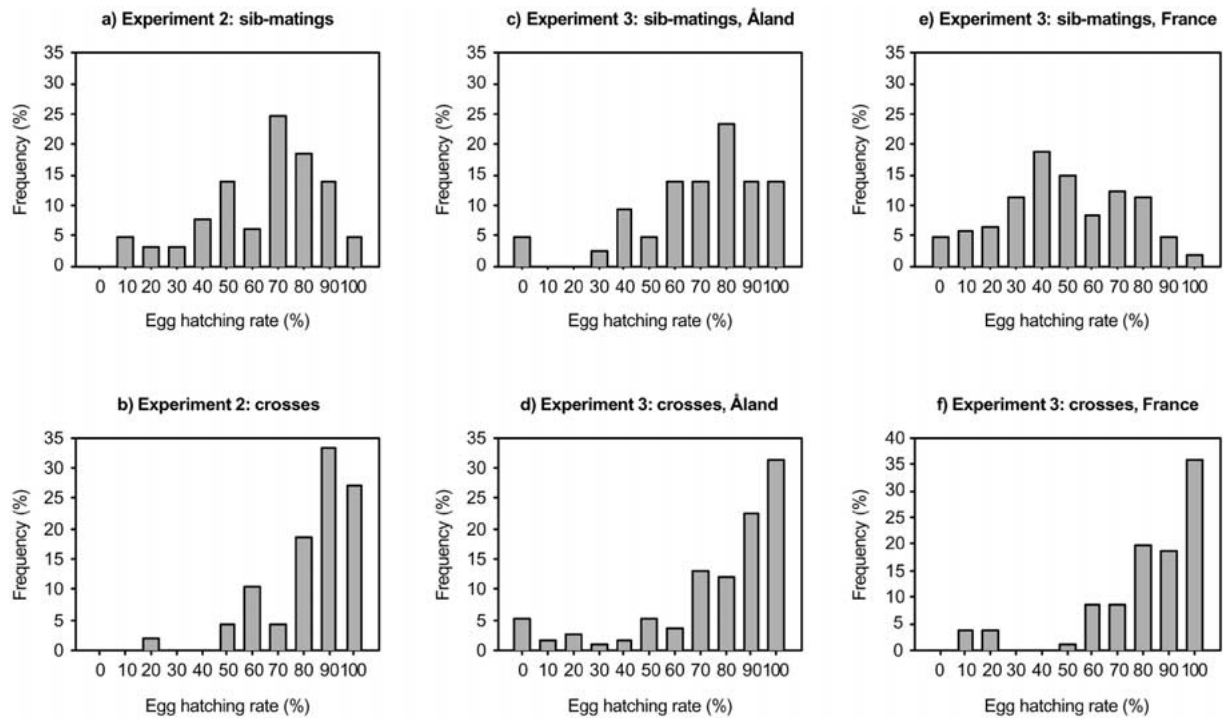


Figure 3. Egg hatching rate (%) in the within-family (a) and between-family/population crosses (b) in Experiment 2, in the within-family (c) and between-population crosses (d) in Åland in Experiment 3, and in the within-family (e) and between-population crosses (f) in France in Experiment 3. Clutches of females with no hatched eggs are excluded.

Table 4. Analysis of egg hatching rate in the Finnish and French metapopulations in Experiment 3. The residual effect was tested against the χ^2 -distribution. The higher level terms were tested against the F -distribution with the mean deviance of the next lower level term as the denominator

Source	Crosses within families			Crosses between populations			Source	France			Source	France		
	Åland			Åland				Åland				France		
	d.f.	Deviance	P	d.f.	Deviance	P	d.f.	Deviance	P	d.f.	Deviance	P		
Popul	5	265.39	0.34	10	466.10	0.58	Cross	9	336.09	0.28	14	674.05	0.17	
Popul/Family	5	180.24	0.31	9	477.83	0.03								
Popul/Family/Female	10	263.16	<0.001	57	1342.06	<0.001	Cross/Female	51	1502.91	<0.001	33	1075.01	<0.001	
Total	20	708.79		76	2285.99		Total	60	1839.00		47	1749.07		

The numbers of females with no eggs hatched ranged from 6 to 16% in the different data sets (Table 2), with no apparent differences between the experiments nor between the treatments. We therefore consider that the majority of these cases was due to random failures in fertilization, though the possibility cannot be excluded that some mated females produced completely infertile eggs.

Larval survival and growth

The relationship between female heterozygosity and several life history traits was studied with regression analysis. Family means of larval survival over diapause were not related to mother's heterozygosity, nor were the larval growth rate during the spring (described by the regression coefficient of family means of larval weight against time, weighting the regression by larval number), the length of the larval period, the survival of larvae to pupation, the pupal weight, or the life span of adult butterflies. There was a

Table 5. Analysis of egg hatching rate in Experiment 3. Treatments are crosses within families versus crosses between families/populations. The residual effect was tested against the χ^2 -distribution. The experiment, treatment, and experiment*treatment terms were tested against (experiment*treatment)/population term

Source	d.f.	Deviance	P
Experiment	1	696.80	<0.001
Treatment	1	2115.75	<0.001
Exp.Treat	1	200.97	0.04
Exp.Treat.Population	38	1741.64	0.03
Residual	165	4841.21	0.00
Total	206	9596.37	

significant positive association between family means of larval weights following diapause and mother's heterozygosity (number of heterozygous loci; regression, $t_{29} = 2.66$, $P = 0.013$) and a significant negative association between the length of the pupal period (family means) and mother's heterozygosity ($t_{23} = -2.24$, $P = 0.035$). Family means of the length of the pupal period were not related to the pupal weight nor to the larval weight after diapause (family means). If corrected for the number of analyses done, none of the results relating to larval growth and survival was significant.

Discussion

Inbreeding depression is often detected in traits related to reproduction (Frankel and Soulé 1981, pp. 59–77). For instance, Saccheri et al. (1996) found a large reduction in egg hatching rate with increasing inbreeding (25% decrease per 10% increase in the level of inbreeding) in the butterfly *Bicyclus anynana*. The decrease in egg hatching rate in F₂ and F₃ in isofemale lines of *B. anynana* was on average 60%, followed by a rapid recovery between F₂ and F₇. In the present study, inbreeding lowered egg hatching rate in all experiments. The average decrease in the crosses within families was 29% when compared to crosses between families/populations. This decrease is less than that found in the study of Saccheri et al. (1996), but it should be noted that in most cases our study included only the zygote component of inbreeding depression, not the fertility component, which is by far the greater component in *B. anynana* (I. Saccheri, unpublished results).

In the natural populations in the Åland Islands, brother-sister matings must represent a common form of inbreeding in *M. cinxia*, imposed on the populations by the spatial metapopulation structure: a large fraction of the small local populations in small habitat patches consists of just a single sib-group of larvae (Hanski et al. 1995a; Hanski 1999, pp. 207–232). If the effect of inbreeding on egg hatching rate is as severe in nature, or even more severe (Crnokrak and Roff 1999), as reported in this study, inbreeding will have important consequences for the performance of small natural populations. Lowered egg hatching rate results in small larval group size, which in turn decreases the survival probability of the larvae in the field (Kuussaari 1998, pp. 65–92). Saccheri et al. (1998) inferred from an observational study on *M. cinxia* and Nieminen et al. (2001) confirmed with an experimental study that inbreeding depression increases extinction risk in small local populations of the butterfly. The findings of the present study suggest that inbreeding depression for egg hatching rate, and the amplification of this negative effect on overall fitness through group size-dependent overwintering survival of larvae (Kuussaari 1998, pp. 65–92), and reduced ability of inbred larval groups to spin a high-quality winter web (Nieminen et al. 2001), are likely to be key contributory factors underlying the observed inbreeding-extinction correlation.

Fewer matings took place in cages with butterflies from small, isolated and most likely more inbred populations than in cages in which the butterflies originated from large, well-connected populations. In their study of natural *M. cinxia* populations, Kuussaari et al. (1998) found that the fraction of mated females increased significantly with increasing population size. In this latter study population size was significantly correlated with population density, and the result was similar when population density was used instead of population size as the explanatory variable. Kuussaari et al. (1998) suggested that reduced mating success at low population density is one mechanism producing an Allee effect, which they also demonstrated for small *M. cinxia* populations. Reduced frequency of matings in small populations could be simply the result of failure to locate a mate, but our results demonstrate that reduced mating success in small populations due to inbreeding may have contributed to the result of Kuussaari et al. (1998). On the other hand, our results do not reveal whether reduced mating success in small populations is due to poor performance of males or females, or both. The

effects of inbreeding on mating ability have not been widely studied. One exception is Sharp (1984), who found that competitive male mating ability is reduced by inbreeding in *Drosophila melanogaster* (see also Partridge et al. 1985).

The decrease in fitness due to inbreeding may be less severe for domesticated species and for otherwise previously inbred or selected lines of experimental organisms than for natural populations. This is because inbreeding exposes deleterious recessives to selection, leading to partial purging of inbred populations (Frankel and Soulé 1981, pp. 59–77; Hedrick 1994). The view that inbreeding depression is always low in partially inbred populations is however not universally accepted. Even in highly inbred populations, inbreeding depression can be severe (Charlesworth and Charlesworth 1987; Hedrick and Kalinowski 2000). For instance, Haag et al. (Haag C, Hottinger J, Riek M, Ebert D, manuscript) found strong inbreeding depression in the presence of competition in a *Daphnia* metapopulation, which points to a high level of genetic load, even though the populations have gone through repeated periods of inbreeding.

The importance of inbreeding in purging deleterious recessives from a population was examined in our study by comparing the effects of inbreeding on egg hatching rate in populations from areas with dissimilar degree of habitat and hence population fragmentation. Local populations in Åland are small and discrete, and have been like that for a long time. In southern France *M. cinxia* is more widespread and has more continuous populations. For these reasons local populations in Åland must have experienced more inbreeding in the past. Our results show that French populations suffer more severe inbreeding depression than populations from Åland, suggesting that deleterious alleles have to some extent been purged from the metapopulation in Åland. This result is important in view of the continuing destruction of natural habitats by human activities, leading to fragmentation of previously large continuous habitats and populations into discrete patches, with local populations susceptible to the harmful effects of inbreeding. Nevertheless, a substantial genetic load remains in the large metapopulation in the Åland Islands. We suggest that migration between small local populations (Hanski et al. 1994; Hanski 1999, pp. 207–232) that carry different deleterious recessives is sufficient for maintaining substantial genetic load in this butterfly metapopulation in spite of the local popula-

tions going repeatedly through population bottlenecks. The relatively short life span of local populations is also expected to limit the purging effect of selection. Richards (2000) has reached the same conclusion for the white campion plant *Silene alba*, which is similarly affected by inbreeding and has a similar metapopulation structure to *M. cinxia* (Hanski 2001). It may also be that part of the inbreeding depression in *Melitaea cinxia* is not due to deleterious recessive alleles that can be purged by inbreeding and selection, but is due to overdominance. In general, however, deleterious recessive alleles are thought to account for most part of the inbreeding depression observed (Charlesworth and Charlesworth 1987).

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