
Effects of Population Size and Food Stress on Fitness-Related Characters in the Scarce Heath, a Rare Butterfly in Western Europe

ANNA CASSEL,* JACK WINDIG,† SÖREN NYLIN,† AND CHRISTER WIKLUND†

*Evolutionary Biology Centre, Department of Conservation Biology and Genetics, Uppsala University, Norbyvägen 18D, S752 36 Uppsala, Sweden, email anna.cassel@ebc.uu.se

†Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden

Abstract: Knowledge about the effects of inbreeding in natural populations is scarce, especially in invertebrates. We analyzed to what extent fitness-related traits in the scarce heath (*Coenonympha hero*), a butterfly, are affected by population size and isolation and whether differences in food quality influence these effects. We categorized nine populations as either large or small and isolated. Full-sib groups of offspring from 27 females were followed under seminatural conditions. Because of increased zygote mortality, egg hatchability was significantly lower in the small and isolated populations than in the large ones. Population category had no effect on larval weight under optimal conditions, but weight was significantly lower in the small-isolated category with low food quality. The effects of inbreeding can thus be hidden when conditions are benign but can appear under stress. Survival also differed significantly between population categories, and larval developmental time tended to be longer in the small-isolated category, irrespective of food conditions. We suggest that the differences in fitness between offspring from large and small isolated populations are at least partly due to inbreeding. This adds a further threat to a species that is already suffering from decreasing population sizes and increasing isolation among populations.

Efectos del Tamaño Poblacional y el Estrés Alimenticio en los Caracteres Relacionados con la Adaptación de la Mariposa *Coenonympha hero*, una Mariposa Rara de Europa Occidental

Resumen: El conocimiento de los efectos de la endogamia en poblaciones naturales es escaso, especialmente en invertebrados. Analizamos el grado al cual las características relacionadas con la adaptación de la mariposa *Coenonympha hero* pueden ser afectadas por el tamaño poblacional y el aislamiento y si las diferencias en la calidad del alimento afectan estos efectos. Categorizamos nueve poblaciones como grandes o pequeñas y aisladas. Grupos de hermanos provenientes de 27 hembras fueron estudiados bajo condiciones seminaturales. Al ser comparada con las poblaciones grandes, la eclosión en las poblaciones pequeñas y aisladas fue significativamente mas baja debido al incremento en la mortalidad de cigotos. La categoría poblacional no tuvo un efecto en el peso larval bajo condiciones óptimas, pero el peso fue significativamente menor en la categoría de las poblaciones pequeñas-aisladas mantenidas con alimentos de baja calidad. Los efectos del cruzamiento cosanguíneo pueden por lo tanto ser ocultados cuando las condiciones son benignas, pero aparecen cuando hay estrés. La supervivencia también difirió significativamente entre las categorías poblacionales y el tiempo del desarrollo larval tendió a ser más largo en la categoría de las poblaciones pequeñas-aisladas independientemente de las condiciones de alimentación. Sugerimos que las diferencias de adaptación entre progenies de poblaciones grandes y poblaciones pequeñas y aisladas son por lo menos parcialmente ocasionadas por la endogamia. Esto agrega una amenaza mas a las especies que ya sufren de disminuciones en sus tamaños poblacionales y de un creciente aislamiento entre sus poblaciones.

Introduction

It has long been debated to what extent inbreeding affects the risk of extinction in natural populations. Lande (1988) argues that demographic processes are more important in the survival of small populations. He suggests that genetic aspects are usually more important in the long-term persistence of a species, mainly by determining the population's ability to adapt to environmental disturbance and change. The effect of genetic factors has gained more attention (Lande 1998). For example, modeling by Lande (1994, 1998) and Noordwijk (1994) indicates that fixation of mildly deleterious mutations may significantly increase the risk of extinction in small populations. Frankham (1995) also points out that a population's sensitivity to demographic and environmental stochasticity is affected by both inbreeding and loss of genetic variation.

The negative effects of inbreeding on fitness (inbreeding depression) are well documented in laboratory experiments in vertebrates, invertebrates, and plants (reviewed by Charlesworth & Charlesworth 1987). Despite the need for information on the effects of inbreeding in natural populations, it is scarce, especially for invertebrates. Recently, Saccheri et al. (1998) showed that reduced heterozygosity is negatively correlated with population growth and survival of local populations of the Glanville fritillary butterfly (*Melitaea cinxia*). They showed that extinction risk increases significantly with decreasing heterozygosity and found significant relationships between heterozygosity and larval survival, adult longevity, and egg-hatching rates, in agreement with expectations following from inbreeding depression (Wright 1977). To our knowledge, this was the first study to demonstrate that natural populations of insects are also affected by inbreeding.

The scarce heath (*Coenonympha hero*; Nymphalidae: Satyrinae) is a small satyrine butterfly found in mosaic landscapes of forest and small-scale agriculture. Their primary habitat is managed hay fields or abandoned arable land, which are characteristically wet, rich in grasses and herbs, and mostly surrounded by forest. Each sub-population is usually well defined and has a locally restricted population structure. Rather than the actual species composition of the vegetation, the climate and structure of the patch appear critical for the species' persistence (Berglund 1996). In central Sweden the adults usually fly from the middle of June until the middle of July. Eggs are laid one by one on dry vegetation, close to the ground and scattered on the patch (A. C., personal observation). The hatched larvae are generalists, feeding on different species of grass. They have been raised successfully in captivity on *Festuca ovina*, *Agrostis capillaris*, and *Dactylis glomerata*. They generally overwinter in the third instar, sitting on the vegetation close to the ground. Growth resumes in early spring, and the fifth instar pupates around the end of May.

The scarce heath is classified as vulnerable in Europe (Heath 1981), is already extinct in some countries, but is still fairly widely distributed in meadows in parts of central Sweden (classified as "near threatened" on the Swedish Red List 2000). The number of known localities is decreasing as more meadows are being abandoned and planted with spruce or become overgrown with shrubs and trees (Berglund 1996). The more-or-less continuous mosaic of meadows in central Sweden has become fragmented, and patches that used to be part of a network now appear isolated. Field studies based on mark-recapture techniques indicate that the scarce heath occurs in locally restricted populations and usually moves only short distances. Even movements between closely situated patches are rare and seem to be highly dependent on corridors such as roads, ditches, and powerline clearings (A.C., unpublished data). Thus, it is likely that even weakly isolated populations lack incoming gene flow. We analyzed to what extent fitness-related traits in the scarce heath are affected by population size and isolation and whether food shortages influence these effects.

Methods

Sampling and Choice of Fitness Characters

For 3 weeks in June 1997, we sampled adult females at random from nine populations in the province of Värmland in central Sweden. We chose these populations based on their size and relative degree of isolation (Table 1), which had been assessed in surveys in 1996 and 1997. The inhabited patches were all rich in grasses and flowering herbs, known to be critical to the species. We divided the selected populations into two groups: one group consisted of six large populations and the other of three populations that were both small and isolated. To confirm the population-size classification, we conducted a more exact population survey in 1998 in all populations. We surveyed 6 days at the peak of the flight period (Table 1). We considered a population small if the calculated population size was <25 individuals for the 6-day survey period, based on Petersen's index as modified by Seber for small sample sizes (Krebs 1999), and we considered a population isolated if the patch lacked a connecting road or any other connective habitat and was situated at least 300 m from the nearest inhabited patch.

On average, we sampled three females from each population (range: 2–5 females). The females were allowed to lay eggs on a tussock of grass, and the eggs were moved to small jars for hatching. Hatchability was calculated as the proportion of hatched eggs of the total number produced per female. To minimize the negative effects of sampling on the small populations, we allowed females to lay a maximum of 60 eggs and then returned

Table 1. Characteristics of studied populations of the scarce heath used for classifying them as large or as small and isolated.

Population classification	Estimated population size (95% confidence interval)	Patch size (10,000 m ²)	Distance to closest population (m)	Connecting road or other suitable corridor structure
Large	73 (32–800)	10	200	yes
Large	74 (38–480)	6.5	100	yes
Large	128 (80–300)	2.5	150	yes
Large	75 (41–271)	1.8	50	yes
Large	53 (30–164)	1	150	yes
Large	51 (20–1200)	0.8	400	yes
Small	10 (8–23)	1	3200	no
Small	7 (2–nc)*	0.8	350	no
Small	15 (7–57)	0.7	500	no

*Not calculated because of lack of recaptures.

them to the sampling site. To further reduce the negative effects of sampling, we returned larvae that were not used to study growth and survival to the site where their mother was collected.

Characters selected to measure fitness were egg hatchability, zygote mortality, larval growth, resistance to food stress, survival, time to pupation, and proportion of crippled adults. All characters relate directly or indirectly to the expected proportion of offspring contributing to the next generation. Slow developmental time can lead to longer exposure to predators and risk of being too small to survive hibernation, and delayed emergency can lead to lost mating opportunities.

Measurements and Growth Conditions

Some eggs did not hatch even though a zygote was clearly visible through the eggshell. We classified them as dead zygotes. To discriminate between fertilized and unfertilized eggs, we observed eggs through a magnifying lens. An egg was classified as unfertilized if no head capsule was visible through the eggshell and the egg remained green.

We placed hatched larvae on tussocks of *Festuca ovina*, known to be a suitable hostplant for the growing larvae (P. O. Wickman, personal observation), kept outdoors under seminatural conditions. A maximum of 30 larvae were chosen at random from each family and split into three groups with 10 larvae on each tussock. To monitor larval survival, we counted the number of larvae in each group before and immediately after winter hibernation. On both occasions we weighed each individual. It was not possible to calculate larval growth for each individual larva because they were kept in groups over the winter. Instead, we calculated growth by subtracting mean weights in each jar in autumn from mean weights in the same jar in spring.

Food quality varied at the end of autumn. We therefore categorized the tussocks as high, medium, and low quality according to the amount of green leaves present. High-quality tussocks contained >90% fresh leaves,

whereas low-quality tussocks contained <10% fresh leaves. The medium tussocks were intermediate in quality. We then compared the survival and growth of larvae within and among the three categories. All tussocks were thereafter changed and checked carefully so that the tussock quality remained high for all larval groups.

In early spring the larvae were put in climate chambers with constant temperature, humidity, and light. Temperature was initially 17° C, and day length 14 hours. We then gradually increased temperature and day length to 21° C and 17 hours. Two days after pupation, we determined the sex and weight of the pupae. Only individuals that eclosed from the pupa successfully were counted as surviving to adulthood. Individuals that did not succeed in extending their wings properly at eclosion were classified as crippled.

Statistical Analyses

We tested differences in egg hatchability, zygote mortality, and proportion of unfertilized eggs with a Mann-Whitney *U* test. Females that produced only unfertilized eggs were excluded from the analyses because it was not clear whether they had mated. We examined the influences of population-size category (large, or small and isolated), food quality (low, medium, high), and their interaction on larval and pupal weights, larval growth, and larval developmental time by analysis of variance (General Linear Model; SAS Institute 1987). Female identity (nominal variable), nested within size, was included in the model as an additional factor. This was done to separate the effect of variation in status among individual females at the time of egg laying (maternal effect) from the effects of food stress and population origin (using type III sum of squares). Because female and male pupae differ in size and development time to pupation, they were analyzed separately.

The survival data were proportional and could not be transformed to normal distributions. Thus, we used stepwise logistic regression (BMDP New System 1994) to examine whether population size, female identity, and

food quality explained significant amounts of variation in the proportion of surviving individuals among the larval groups at different stages of the lifecycle. Step selection was based on maximum-likelihood ratios, and we used a maximum of 100 iterations. The critical p value for entering a variable into the regression model was set to 0.15, and the p value for having a variable removed was set to 0.20, according to Hosmer and Lemeshow (1989). Variance in the proportion of crippled adults among the groups was analyzed in the same way.

Results

Twenty-seven of the 31 sampled females (9 from small, isolated populations and 22 from large) produced up to 61 eggs each. The remaining four females survived at least 2 days in captivity but produced no fertile eggs; they were most likely unmated when captured, because no spermatophore were found in their bursae when it was dissected. Three of the females originated from small, isolated populations, which is a significantly higher proportion than was found for the large populations ($\chi^2 = 4.71$, $p = 0.03$).

Despite the low number of populations and families in the study, hatchability was significantly lower in small, isolated populations than in large populations ($r = 0.023$, Mann-Whitney U test; Fig. 1). The difference was due to higher zygote mortality in the small, isolated populations: ($p = 0.0025$). The proportion of unfertilized eggs did not differ significantly from that of the large populations ($p = 0.3364$; Fig. 1).

The effect of population size on larval weight was significant, despite the large statistical effect of individual females (Table 2). The statistical effects of female and food quality were weaker in spring than in autumn, whereas the statistical strength of population size remained. Furthermore, there was a highly significant interaction between population size and food quality (Table 2). In the high- and medium-quality tussocks there was no difference in larval weight between the large and the small and isolated populations, whereas weights on the low-quality tussocks were lower for larvae from small and isolated populations compared with those of larvae from large populations (Fig. 2). The effect of former differences in food quality on larval weights in spring was not significant (Table 2). There was a highly significant effect of family origin on larval developmental time ($F = 6.32$, $p = 0.0001$), but neither population size nor food quality had any significant effect ($F = 2.11$ and 0.17 , $p = 0.150$ and 0.847 , respectively). However, there was a trend toward longer development time in small and isolated populations (Fig. 3).

There was no observable effect of former differences in food quality on pupal weight or on proportion of crippled

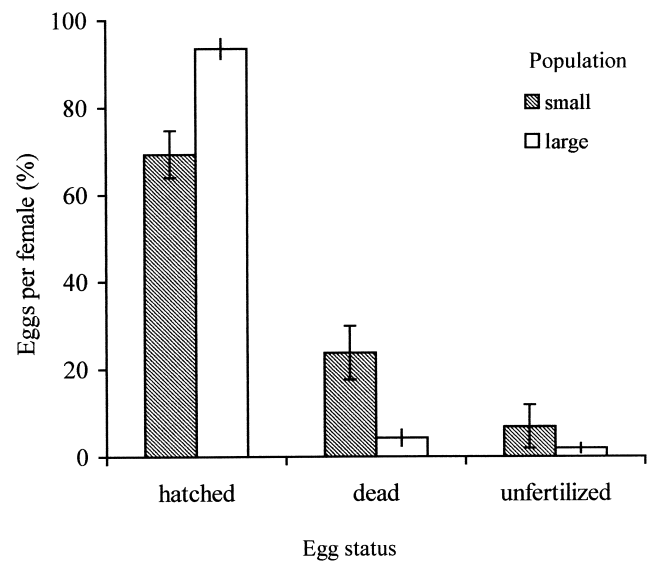


Figure 1. Percentage of total number of eggs per female (± 1 SE) that hatched, died as zygotes, or remained unfertilized in small and isolated populations and in large populations.

pled versus successfully eclosed adults. Pupal weight also did not differ between the small and isolated and the large populations.

Survival from egg to adulthood was 19% for offspring from small populations and 31% for large populations. The effect of female identity was highly significant at both larval stages, at pupation, and at eclosion, indicating a significant (genetic and/or nongenetic) maternal effect. Population size was significant at all time steps except at eclosion, and former differences in food quality were close to significant in spring and significant at pupation and eclosion (Table 3).

Table 2. Analysis of variance (GLM; SAS Institute 1987) for larval weights (mg) of offspring from females originating from large and small populations of the scarce heath measured before and after hibernation (autumn and spring, respectively).

Factors of variation*	df	ms	F	p
Autumn ($n = 483$)				
Population size (N)	1	11.888	5.46	0.020
Female (f)	25	22.127	10.16	>0.001
Food quality (q)	2	24.122	11.08	>0.001
$N \times q$	2	32.663	15.00	>0.001
$f \times q$	17	13.316	6.12	>0.001
Total	47	22.887	10.51	>0.001
Spring ($n = 376$)				
Population size (N)	1	13.787	7.14	0.008
Female (f)	25	10.118	5.24	>0.001
Food quality (q)	2	4.515	2.34	0.098
$N \times q$	2	6.175	3.20	0.042
$f \times q$	13	9.392	4.87	>0.001
Total	43	9.870	5.12	>0.001

*The n is total number of larvae measured. Population size is small or large; food quality (q) is high, medium, or low.

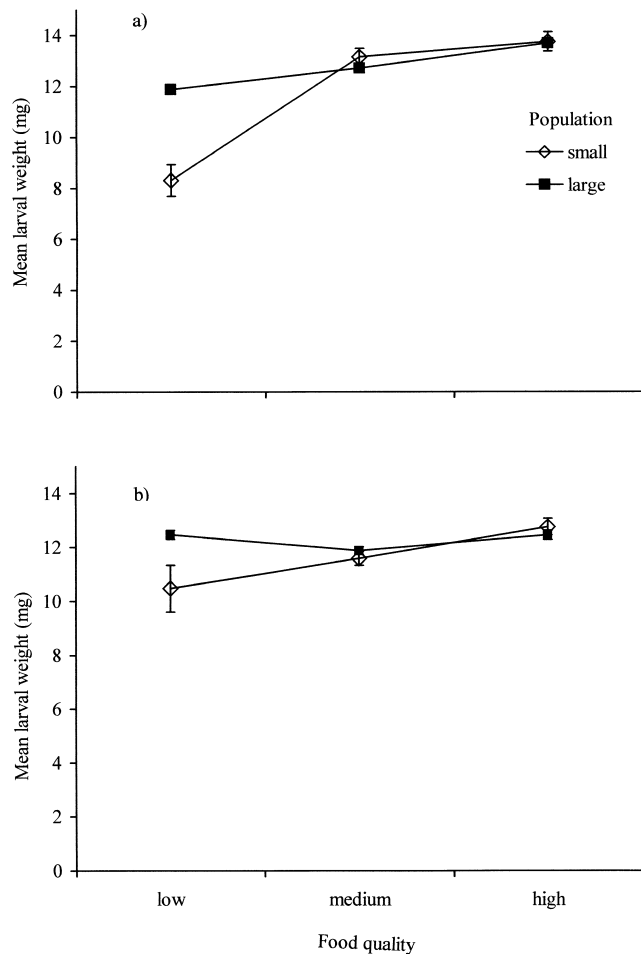


Figure 2. Mean larval weight (± 1 SE) of individuals belonging to the same larval group in (a) autumn and (b) spring, grouped by population size and food quality.

Discussion

Because of higher zygote mortality, eggs of females from small, isolated populations were significantly less hatchable than those from large populations. Furthermore, when food quality was low, larvae gained less weight and were less likely to survive. Also, there was a tendency toward longer developmental time from egg to pupation than among offspring from large populations. These observed differences agree with the expectations following from a higher level of inbreeding in small and isolated populations (Wright 1977; Allendorf & Leary 1986), but some of the differences may have been caused by undetected differences in patch quality. For example, a large patch can, as a consequence of being larger, include more plants suitable as nectar sources. A large patch may also include a larger variety of microhabitats that will buffer against environmental variation (Kindvall 1996). Such differences can be reflected in a female's offspring through maternal effects.

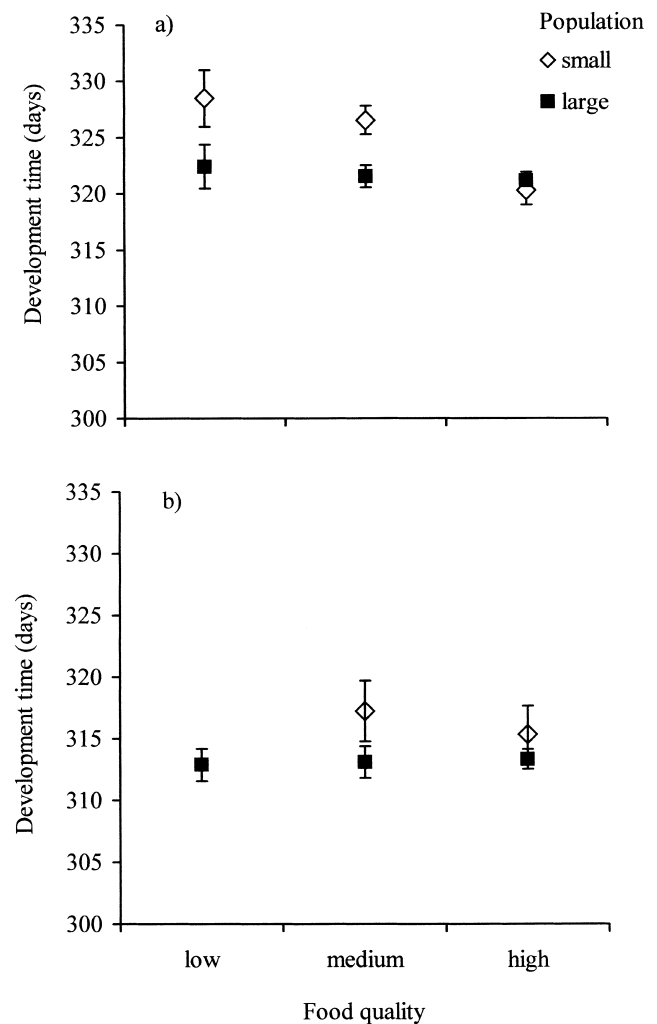


Figure 3. Total larval development time (± 1 SE) for (a) females and (b) males. Because none of the larvae from the small population that experienced low food quality developed into a male, this group is absent from the figure.

It seems unlikely, however, that patch quality is solely responsible for the observed differences between small and large populations. In that case we would expect the influence of both population size and individual females to decrease from autumn to spring, because all larvae were kept and fed in the same environment. Instead, maternal influence decreased, and population size explained a larger part of the variation in larval weights. Inbreeding experiments on other butterflies have demonstrated similar effects on larval performance, although the magnitude of effect differs between species (Di Mare & Araújo 1986; Haag & Araújo 1994; Orr 1994; Saccheri et al. 1996; Saccheri et al. 1998).

A possible way to separate the effects of inbreeding from those of environment is to perform reciprocal crossings between individuals from the studied popula-

Table 3. Assessment of variance components in proportion of surviving offspring of each female explained by population size (large or small), female gender (nominal variable), and food quality (low, medium, or high) at different stages in the lifecycle of the scarce heath through stepwise logistic regression (BMDP New System 1994).*

<i>Variables analyzed</i>	<i>df</i>	<i>Improvement chi-square</i>	<i>P</i>
Autumn			
size	1	3.86	0.050
female	26	315.52	0.000
food quality	1	0.73	0.393
constant	1	0.00	1.000
Spring			
size	1	6.18	0.013
female	26	136.97	0.000
food quality	1	3.15	0.076
constant	1	0.00	1.000
Pupation			
size	1	7.36	0.007
female	26	77.27	0.000
food quality	1	6.36	0.012
constant	1	0.00	1.000
Eclosion			
size	1	0.00	1.000
female	26	54.60	0.001
food quality	1	5.25	0.022
constant	1	83.30	0.000
Crippled			
size	1	0.00	0.986
female	26	39.08	0.048
food quality	1	0.82	0.366
constant	1	657.57	0.000

*Statistics pertain to the final logistic-regression model. The proportion of crippled adults was analyzed by the same method.

tions. If inbreeding were responsible for the poor performance of the individuals originating from the small and isolated populations, within-population crossings, especially those within small and isolated populations, would result in offspring showing similar or worse performance. On the other hand, crossings with individuals from different small populations would result in offspring performing better than both parental generations. If environmental factors alone explain the observed differences, one would not expect such differences in performance between the within- and across-population crossings. An alternative to crossing experiments is to use highly variable genetic markers to reveal whether there really are differences in genetic variability and heterozygosity among the studied populations.

Food stress affected growing larvae differently. There was a clear difference in performance between the two types of populations when they were reared under optimal and stressful conditions before hibernation (Fig. 2a). The similar larval weights in the high- and medium-quality groups indicate that tussocks of both of these qualities supported the growth of larvae well. Low-quality tussocks, on the other hand, affected the growth of lar-

vae from small and isolated populations significantly more than was the case for larvae from large populations, resulting in low weights in the autumn. These low weights persisted in the spring, following hibernation (Fig. 2b). At pupation, however, there was no remaining effect of former differences in food quality on either pupal weight or larval developmental time. Consequently, this category of larvae apparently was able to compensate for the bad start by growing faster when all larvae were given fresh grass. It is known that satyrine larvae do not normally maximize growth rates and, as a consequence, they can adjust their rate of growth to environmental conditions and their own state (e.g., Nylin et al. 1993, 1996; Gotthard 1998). High growth rates are probably associated with costs, such as increased risk of mortality from starvation or predation (Wickman et al. 1990; Gotthard et al. 1994; Nylin & Gotthard 1998). If so, food stress places further burdens on the already sensitive small populations. A short drought may not have a lasting effect on the survival and development of small and isolated populations, but a longer period of stress may significantly reduce life expectancy.

Our study emphasizes the importance of performing inbreeding experiments not only under favorable conditions but also under stress, which commonly occurs in natural populations. Several studies have shown that differences in both environment and stress levels can cause differences in the expression of inbreeding (Chen 1993; Heschel & Paige 1995; Jiménez et al. 1994; Pray et al. 1994). It is therefore likely that the effect of stress our results suggest may be even more severe under natural conditions, when different forms of stress can coincide.

Low population densities and isolation may result in matings between close relatives and in a reduction of mating opportunities (Lande 1988). Kuussaari et al. (1998) found that females from small, isolated populations have significantly reduced mating success compared with females from large, dense populations. Our limited data point in the same direction: three out of the four unmated females originated from small and isolated populations. This indicates that in these small populations males and females may have difficulty finding mates.

Offspring of females from small, isolated populations in our study were less successful than offspring of females from large populations. We suggest that this is at least partly due to inbreeding. The marked reduction in egg hatchability, the increased susceptibility of larvae to food stress, the lower survival, increased mating failures, and longer development time most likely will have a negative effect on the persistence of these populations. To some extent, larvae were able to compensate for a limited exposure to stress. Nevertheless, our results indicate that the scarce heath is sensitive to inbreeding and that populations located as close as 300 m to another inhabited patch can lack necessary incoming gene flow when connecting corridors are missing.

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Literature Cited

- Allendorf, F.W., and R.F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. Pages 57-76 in M. E. Soulé, editor. *Conservation biology: the science of scarcity and diversity*. Sinauer Associates, Sunderland, Massachusetts.
- BMDP New System. 1994. Statistical software. BMDP New System, Cork, Ireland.
- Berglund, S.-Å. 1996. *Coenonympha bero* Linné 1761, scarce heath. Species fact sheet. Threatened Species Unit, Uppsala, Sweden. (In Swedish.)
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**:237-268.
- Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L.) (land snail). *Heredity* **71**:456-461.
- Di Mare, R. A., and A. M. Araújo. 1986. A first survey of inbreeding effects in *Heliconius erato phyllis* (Lepidoptera; Nymphalidae). *Revista Brasileira de Genética* **IX** 1:11-20.
- Frankham, R. 1995. Conservation genetics. *Annual Review of Genetics* **29**:305-327.
- Gotthard, K. 1998. Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: investigating an adaptive reaction norm. *Journal of Evolutionary Biology* **99**:281-289.
- Gotthard, K., S. Nylin, and C. Wiklund. 1994. Adaptive variation in growth rate: life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. *Oecologia* **99**:281-289.
- Haag, K. L., and A. M. Araújo. 1994. Inbreeding, genetic load and morphometric variation in natural populations of *Dryas iulia* (Lepidoptera, Nymphalidae). *Revista Brasileira de Genética* **IX** 17:35-39.
- Heath, J. 1981. Threatened Rhopalocera (butterflies) in Europe. European Committee for the Conservation of Nature and Natural Resources, Huntingdon, England.
- Heschel, M. S., and K. N. Paige. 1995. Inbreeding depression, environmental stress, and population size variation in scarlet gilia (*Ipomopsis aggregata*). *Conservation Biology* **9**:126-133.
- Hosmer, D. W., Jr., and S. Lemeshow. 1989. *Applied logistic regression*. Wiley, New York.
- Jiménez, J. A., K. A. Hughes, G. Alaks, L. Graham, and R. C. Lacy. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* **266**:271-273.
- Kindvall, O. 1996. Habitat heterogeneity and survival in a bush cricket metapopulation. *Ecology* **77**:207-214.
- Krebs, C. J. 1999. Estimating abundance: mark-recapture techniques. Pages 20-35 in *Ecological methodology*. 2nd edition. Addison Wesley Longman, Menlo Park, California.
- Kuussaari, M., I. Saccheri, M. Camara, and I. Hanski. 1998. Allée effect and population dynamics in the Glanville fritillary butterfly. *Oikos* **82**:384-392.
- Lande, R. 1988. Genetics and demography in biological conservation. *Science* **241**:1455-1460.
- Lande, R. 1994. Risk of population extinction from fixation of new deleterious alleles. *Evolution* **48**:1460-1469.
- Lande, R. 1998. Risk of population extinction from fixation of deleterious and reverse mutations. *Genetica* **102/103**:21-27.
- Noordwijk, A. J. 1994. The interaction of inbreeding depression and environmental stochasticity in the risk of extinction of small populations. Pages 131-146 in V. Loeschcke, J. Tomiuk, and S. K. Jain, editors. *Conservation genetics*. Birkhäuser Verlag, Basel, Switzerland.
- Nylin, S., and K. Gotthard. 1998. Plasticity in life-history traits. *Annual Review of Entomology* **43**:63-83.
- Nylin, S., C. Wiklund, P. O. Wickman, and E. Garcíabarrós. 1993. Absence of trade-offs between sexual size dimorphism and early male emergence in a butterfly. *Ecology* **74**:1414-1427.
- Nylin, S., K. Gotthard, and C. Wiklund. 1996. Reaction norms for age and size at maturity in *Lasiommata* butterflies: predictions and tests. *Evolution* **50**:1259-1264.
- Orr, A. G. 1994. Inbreeding depression in Australian butterflies: some implications for conservation. *Memoirs of the Queensland Museum* **36**:179-184.
- Pray, L. A., J. M. Schwartz, C. J. Goodnight, and L. Stevens. 1994. Environmental dependency of inbreeding depression: implications for conservation biology. *Conservation Biology* **8**:562-568.
- Saccheri, I. J., P. M. Brakefield, and R. A. Nichols. 1996. Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution* **50**:2000-2013.
- Saccheri, I., M. Kuussaari, M. Kankare, P. Vikman, W. Fortelius, and I. Hanski. 1998. Inbreeding and extinction in a butterfly population. *Nature* **392**:491-494.
- SAS Institute. 1987. *Statistics*. Version 6.03. SAS Institute, Cary, North Carolina.
- Wickman, P. O., C. Wiklund, and B. Karlsson. 1990. Comparative phenology of four satyrine butterflies inhabiting dry grasslands in Sweden. *Holarctic Ecology* **13**:238-246.
- Wright, S. 1977. Inbreeding in animals: differentiation and depression. Pages 44-96 in *Evolution and the genetics of populations*. University of Chicago Press, Chicago.

