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Research article

Has inbreeding depression led to avoidance of sib mating in the Glanville fritillary butterfly (*Melitaea cinxia*)?

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Abstract. Previous work on the Glanville fritillary butterfly (*Melitaea cinxia*) shows substantial inbreeding depression in both of our two study regions, Finland and southern France. The influence of inbreeding depression on population dynamics should depend on the strength of inbreeding avoidance. We conducted mate choice experiments to ascertain whether and to what extent butterflies avoid mating with their sibs. Experiments with similar design were done in the laboratory with Finnish butterflies and in the field with French butterflies. Each female was given a choice of mates with equal opportunity to mate with a sib or with a non-sib. In neither experiment was there a trend towards avoidance of sib mating. 95% confidence intervals for the proportion of non-sib matings were 12–62% in the laboratory experiment and 28–69% in the field experiment. Any preference for non-sibs must be slight, and can provisionally be ignored in modelling the dynamics of *M. cinxia* populations.

Key words: inbreeding avoidance, inbreeding depression, mate choice, *Melitaea cinxia*, metapopulation

Introduction

Whenever one finds inbreeding depression in a species one is tempted to expect that the species should possess some mechanisms to promote outbreeding. Such mechanisms are well-known in both plants and animals (Crawley, 1997; Futuyma, 1997: p. 622). For instance, a frequent mechanism in animals is preference for non-relatives as mates. When such a preference exists, a quantitative estimate of its strength is necessary to incorporate comprehensively the effects of inbreeding depression into population dynamic models. It is in this spirit that we undertook the present study to estimate tendencies for matings to occur between sibs and non-sibs in a species that suffers substantial inbreeding depression and has been the focus of intensive population dynamic study.

Our study organism is the Glanville fritillary butterfly, *Melitaea cinxia*. We have studied populations from two geographical regions: the Åland Islands, Finland, and Languedoc-Roussillon, France. The Finnish system has been the subject of long-term study. It comprises a large metapopulation in which population extinctions, colonisations and bottlenecks have been frequent (Hanski, 1999: pp. 207–232). Local populations are typically small, usually less than 10 larval groups and often only one, in which case it is almost certain that all individuals in the patch are siblings (Hanski *et al.*, 1995a; Hanski, 1999: pp. 207–232). Females lay several egg clutches of approximately 100–200 eggs each (Kuussaari, 1998; Hanski, 1999: pp. 207–232). The hatched sib-groups of larvae live gregariously in a communal web (Kuussaari, 1998). Colonisations are often accomplished by single mated females. Due to this population structure, there are regular opportunities for brother–sister mating.

Given the population structure of *M. cinxia* in the Åland Islands, one might expect that deleterious recessive alleles have been purged and there would be no inbreeding depression. However, this is not the case: inbreeding depression has not been purged, it is substantial, affecting egg hatching rates and larval survival, even causing population extinctions in the field (Saccheri *et al.*, 1998; Haikola *et al.*, 2001; Nieminen *et al.*, 2001). In previous work the rate of successful mating was lower among butterflies originating from small and isolated, and hence presumably more inbred populations, than among butterflies from large and well-connected populations (Haikola *et al.*, 2001). These results did not demonstrate any underlying mechanism. This mechanism could lie in poor performance of either inbred males or females. Inbred individuals could be less attractive to the opposite sex in general, and/or inbred males could suffer low mating success from reduced agility (Joron and Brakefield, 2003). Regardless of its mechanistic basis, increased mating ability of outbred individuals could strengthen the ‘genetic rescue’ effect – restoration of genetic variation and alleviation of inbreeding depression in small local populations due to immigration of outbred individuals (Thrall *et al.*, 1998; Ball *et al.*, 2000; Hedrick and Kalinowski, 2000; Richards, 2000; Ingvarsson, 2001).

The purpose of the present study was to ask whether inbreeding avoidance occurs in Finnish and/or in French populations of *M. cinxia*. Inbreeding depression is strong in both areas (Haikola *et al.*, 2001). We conducted mate choice experiments in the laboratory with Finnish butterflies and in the field with French butterflies to find out whether they avoided mating with siblings.

Methods

Laboratory experiments

We gathered larval groups for the experiment soon after they had broken diapause in spring 2000 and 2001. To minimise population-specific effects we sampled widely across the metapopulation. We assume that larvae from the same group were siblings, because Finnish *M. cinxia* remain gregarious throughout larval life (Kuussaari, 1998). Anastomosis of larval groups does occur (Palo *et al.*, 1995), so that as time passes it becomes less certain that groups found in the field comprise uncontaminated sets of siblings. However, as the female butterflies and their presumed brothers in the cages where mating took place were in the vast majority of cases from populations that had just one larval group in the previous autumn (M. Nieminen, personal communication), the assumption of uncontaminated sib groups is very unlikely to be violated.

We raised the larvae to adults, which were marked individually and fed with mild honey water. Mating trials were conducted indoors in cylindrical net cages (diameter 41 cm, height 47 cm) under artificial light at temperatures of ca 25°–35° and rH 25–65%. Each virgin female was caged with four males, comprising two sibs and two non-sibs taken from a different population. We controlled for male age as well as possible: the mean age of the brothers of the females at the time when they were placed in the cage was 3.0 days and that of the non-sib males was 4.2 days. We observed the cages frequently and recorded the first mating that occurred in each cage. The results are for matings in 15 mating cages, with butterflies from 24 different populations. All mated females were from a different population.

Field experiments

Eggs were obtained from field-caught females in a large (>100 adults) population of *M. cinxia* 2 km north of Pompignan in the région Languedoc–Roussillon in April 2000 and 2001. Larval groups were raised on their natural host, *Plantago lanceolata*, and became adults about 8 weeks after oviposition. We chose a natural habitat containing adequate nectar supply in which to release butterflies. Using only those families that had produced at least 12 healthy males simultaneously, we released marked males from two families at a time, using an equal number of males from each family. After a few minutes the released males formed a temporary population with apparently normal behaviour. We then released virgin females, one at a time, into a population of males, half of which were sibs of the female and half non-sibs. We followed each female and noted the identity of the male with which she mated. We did this with 10 families, released as five pairs of families. We used the same site for all the experiments and waited a few days between each trial, so that the butterflies released previously were no longer present.

The design of the field experiment appears, by releasing females one at a time, to offer males a sequential choice of sib and non-sib females while offering each female a simultaneous choice of sib and non-sib males. However, the released females normally encountered males one at a time, so in practice we administered sequential choice tests to both males and females. Strong mating preferences on the part of either sex should be about equally likely to influence the data.

Results

We observed 15 matings in the laboratory experiment and 25 in the field experiment. In both experiments each female had an equal opportunity to mate with a sib or a non-sib. Results are shown in Table 1. Neither of the experiments showed a trend for avoidance of sib mating. Granted our limited sample sizes it is appropriate to calculate 95% confidence intervals for the proportion of non-sib matings to ask what strength of inbreeding avoidance might exist in our experiments (Colegrave and Ruxton, 2003). 95% confidence intervals (exact binomial test, *R* 1.6.1) for the proportion of non-sib matings were 12–62% in the laboratory experiment and 28–69% in the field experiment (Table 2).

Discussion

Avoidance of inbreeding appears to be widespread among animals (Koenig and Pitelka, 1979; Hoogland, 1982; Cockburn *et al.*, 1985; Krackow and

Table 1. Numbers of females mating with brothers and with unrelated males

Experiment	Sib matings	Non-sib matings
Laboratory	10	5
Field	13	12

Table 2. 95% confidence intervals for the proportion of non-sib matings in the laboratory and field experiments

Experiment	Lower	Upper
Laboratory	12%	62%
Field	28%	69%

Matuschak, 1991; Dobson *et al.*, 1997). Simmons (1991) found that the time spent by female field crickets, *Gryllus bimaculatus*, with courting males, the probability of mating, the time spent with a guarding male after mating, and the duration of spermatophore attachment all increased with decreasing degree of relatedness with the male. Keller and Passera (1993) similarly found a preference for mating with unrelated males as opposed to brothers in female *Iridomyrmex humilis* ants. There is also evidence for 'optimal outbreeding,' where individuals of intermediate relatedness are preferred as mates (Bateson, 1978, 1982; Keane, 1990; Peacock and Smith 1997). In contrast to these observations, Keane *et al.* (1996) and Gibbs and Grant (1989) found no evidence of inbreeding avoidance in the dwarf mongoose, *Helogale parvula*, and Darwin's Medium Ground Finch, *Geospiza fortis*, respectively. Some animals show post-copulatory mate choice such as preferential use of sperm from non-sibs and higher frequency of re-mating after mating with a sib. At least in some cases this process is known to favour outbreeding (Olsson *et al.*, 1996; Markow, 1997). It remains to be ascertained whether *M. cinxia* indulge in post-copulatory avoidance of inbreeding. We found no evidence that mechanisms to avoid inbreeding have evolved in either the French or the Finnish butterflies. Local populations of *M. cinxia* in the Åland Islands in Finland are often small and relatively isolated. Small population size is the condition under which inbreeding is most likely and the payoff for inbreeding avoidance is highest. However, at the same time it is the condition under which rejection of a sib as a mate carries the highest cost in terms of risking failure to mate at all. Indeed, Kuussaari *et al.* (1998) found a significant increase in the fraction of mated females with increasing population size/density.

In the very small Finnish populations it must often happen that all the butterflies emerging in a habitat patch are siblings and that butterflies would need to emigrate in order to avoid sib mating. Female *M. cinxia* are known to emigrate in response to failing to find their most-preferred host plants for oviposition (Hanski and Singer, 2001), hence it is conceivable that they might likewise emigrate in response to failing to find their preferred mates. However, in the light of our experimental result that any preference for mating with non-sibs must be weak at best, we can now conclude that the frequent opportunities for sib mating among Finnish butterflies are indeed likely to translate into frequent actual sib matings. A corollary is that emigration is an unlikely response to failure to locate non-sibs as potential mates. On the other hand, it is known that the rate of emigration in *M. cinxia* is elevated when density is low (Kuussaari *et al.*, 1996), which would reduce sib mating.

Immigration of new individuals and their breeding in small inbred populations of *M. cinxia* would certainly alleviate the negative consequences of inbreeding in these populations. However, this study suggests that any such 'genetic rescue' would not be strengthened by increased mating success of unrelated immigrants over related resident males and would have a weaker influence on the population dynamics of *M. cinxia* than the demographic rescue effect that has been demonstrated in the Åland metapopulation (Hanski *et al.*, 1995b; Hanski, 1999: p. 141–157). One caveat that still remains stems from our previous result (Haikola *et al.*, 2001) of lower rate of successful mating among butterflies originating from small and isolated, and hence presumably more inbred populations, than among butterflies from large and well-connected populations. We do not know the basis of this result. If it was at least partly due to reduced mating ability of inbred males, then immigration from a large source population might produce a stronger genetic rescue effect than that expected from just the reduced frequency of inbred individuals in the small local population.

In conclusion, any tendency towards inbreeding avoidance in *M. cinxia* must be weak. Models of metapopulation dynamics in *M. cinxia* should not go far astray if they use a random mating assumption to predict frequency of sib matings. It would indeed be useful to include the experimentally estimated inbreeding depression into models of metapopulation dynamics, as has already been done in the case of a *Silene alba* metapopulation (Thrall *et al.*, 1998).

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References

Ball, S.J., Adams, M., Possingham, H.P. and Keller, M.A. (2000) The genetic contribution of single male immigrants to small, inbred populations: a laboratory study using *Drosophila melanogaster*. *Heredity* **84**, 677–684.

Bateson, P. (1978) Sexual imprinting and optimal outbreeding. *Nature* **273**, 659–660.

Bateson, P. (1982) Preferences for cousins in Japanese quail. *Nature* **295**, 236–237.

Cockburn, A., Scott, M.P. and Scotts, D.J. (1985) Inbreeding avoidance and male-biased natal dispersal in *Antechinus* spp. (Marsupialia: Dasyuridae). *Anim. Behav.* **33**, 908–915.

Colegrave, N. and Ruxton, G.D. (2003) Confidence intervals are a more useful complement to nonsignificant tests than are power calculations. *Behav. Ecol.* **14**, 446–447.

Crawley, M.J. (1997) Sex. In M.J. Crawley (ed.) *Plant Ecology*. 2nd edn. Blackwell Science, pp. 156–213.

Dobson, F.S., Chesser, R.K., Hoogland, J.L., Sugg, D.W. and Foltz, D.W. (1997) Do black-tailed prairie dogs minimize inbreeding? *Evolution* **51**, 970–978.

Futuyma, D.J. (1997) *Evolutionary Biology*. 3rd edn. Sinauer Associates, Sunderland, Massachusetts.

Gibbs, H.L. and Grant, P.R. (1989) Inbreeding in Darwin's Medium Ground Finches (*Geospiza fortis*). *Evolution* **43**, 1273–1284.

Haikola, S., Fortelius, W., O'Hara, R.B., Kuussaari, M., Wahlberg, N., Saccheri, I.J., Singer, M.C. and Hanski, I. (2001) Inbreeding depression and the maintenance of genetic load in *Melitaea cinxia* metapopulations. *Conserv. Genet.* **2**, 325–335.

Hanski, I. (1999) *Metapopulation Ecology*. Oxford University Press, Oxford.

Hanski, I., Pakkala, T., Kuussaari, M. and Lei, G. (1995a) Metapopulation persistence of an endangered butterfly in a fragmented landscape. *Oikos* **72**, 21–28.

Hanski, I., Pöyry, J., Pakkala, T. and Kuussaari, M. (1995b) Multiple equilibria in metapopulation dynamics. *Nature* **377**, 618–621.

Hanski, I. and Singer, M.C. (2001) Extinction-colonization dynamics and host-plant choice in butterfly metapopulations. *Am. Nat.* **158**, 341–353.

Hedrick, P.W. and Kalinowski, S.T. (2000) Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst.* **31**, 139–162.

Hoogland, J.L. (1982) Prairie dogs avoid extreme inbreeding. *Science* **215**, 1639–1641.

Ingvarsson, P.K. (2001) Restoration of genetic variation lost – the genetic rescue hypothesis. *Trends Ecol. Evol.* **16**, 62–63.

Joron, M. and Brakefield, P.M. (2003) Captivity masks inbreeding effects on male mating success in butterflies. *Nature* **424**, 191–194.

Keane, B. (1990) The effect of relatedness on reproductive success and mate choice in the white-footed mouse, *Peromyscus leucopus*. *Anim. Behav.* **39**, 264–273.

Keane, B., Creel, S.R. and Waser, P.M. (1996) No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. *Behav. Ecol.* **7**, 480–489.

Keller, L. and Passera, L. (1993) Incest avoidance, fluctuating asymmetry, and the consequences of inbreeding in *Iridomyrmex humilis*, an ant with multiple queen colonies. *Behav. Ecol. Sociobiol.* **33**, 191–199.

Koenig, W.D. and Pitelka, F.A. (1979) Relatedness and inbreeding avoidance: counterploys in the communally nesting acorn woodpecker. *Science* **206**, 1103–1105.

Krackow, S. and Matuschak, B. (1991) Mate choice for non-siblings in wild house mice: evidence from a choice test and a reproductive test. *Ethology* **88**, 99–108.

Kuussaari, M. (1998) *Biology of the Glanville fritillary butterfly* (*Melitaea cinxia*). PhD thesis, University of Helsinki, Finland.

Kuussaari, M., Nieminen, M. and Hanski, I. (1996) An experimental study of migration in the Glanville fritillary butterfly *Melitaea cinxia*. *J. Anim. Ecol.* **65**, 791–801.

Kuussaari, M., Saccheri, I., Camara, M. and Hanski, I. (1998) Allee effect and population dynamics in the Glanville fritillary butterfly. *Oikos* **82**, 384–392.

Markow, T.A. (1997) Assortative fertilization in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **94**, 7756–7760.

Nieminen, M., Singer, M.C., Fortelius, W., Schöps, K. and Hanski, I (2001) Experimental confirmation that inbreeding depression increases extinction risk in butterfly populations. *Am. Nat.* **157**, 237–244.

Olsson, M., Shine, R., Madsen, T., Gullberg, A. and Tegelström, H. (1996) Sperm selection by females. *Nature* **383**, 585.

Palo, J., Varvio, S.-L., Hanski, I. and Väinölä, R. (1995) Developing microsatellite markers for insect population structure: complex variation in a checkerspot butterfly. *Hereditas* **123**, 295–300.

Peacock, M.M. and Smith, A.T. (1997) Nonrandom mating in pikas *Ochotona princeps*: evidence for inbreeding between individuals of intermediate relatedness. *Mol. Ecol.* **6**, 801–811.

Richards, C.M. (2000) Inbreeding depression and genetic rescue in a plant metapopulation. *Am. Nat.* **155**, 383–394.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. and Hanski, I. (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.

Simmons, L.W. (1991) Female choice and the relatedness of mates in the field cricket, *Gryllus bimaculatus*. *Anim. Behav*. **41**, 493–501.

Thrall, P.H., Richards, C.M., McCauley, D.E. and Antonovics, J. (1998) Metapopulation collapse: the consequences of limited gene-flow in spatially structured populations. In J. Bascompte and R.V. Solé (eds.) *Modeling spatiotemporal dynamics in ecology*. Springer-Verlag, Berlin, pp. 83–104.