May 2009

Captive breeding causes small body size in Morpho peleides limpida (Nymphalidae : Morphinae)

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ABSTRACT

Captive breeding has many positive implications in terms of conservation, but when practiced irresponsibly, can result in harmful consequences like inbreeding. Inbreeding has been shown to result in developmental instabilities like small body size and fluctuating asymmetry. In this study, I used small body size as a proxy for inbreeding by comparing measurements of the butterfly *Morpho peleides limpida* from wild and captive bred populations in terms of right forewing and right antennae length. Both measures of size were found to be larger in wild populations than captive populations. The greatest variation in the means was observed in female right wing measurements, with larger mean lengths in wild populations (76.99mm ± 4.23mm) compared to captive populations (66.58mm ± 2.10mm). This shows that rearing techniques used in butterfly gardens may invoke varying amounts of inbreeding. New measures must be taken to practice more responsible breeding such as ensuring the introduction and maintenance of more genetic variation in captive populations.

RESUMEN

La cría en cautiverio tiene muchas implicaciones positivas para conservacion, pero cuando la practican con irresponsabilidad, puede resultar en consequencias dañinas como endogamia. La endogamia puede resultar en inestabilidades en el desarrollo como un cuerpo pequeño y asimetría fluctuante. En esto estudio, yo usé el cuerpo pequeño como un aproximado de endogamia comparando medidas del largo del ala derecha y el largo de la antena. Las dos medidas de tamaño fueron mayores en las mariposas *Morpho peleides limpida* en la naturaleza. La mayor diferencias observadas fueron en las alas derechas de las hembra, siendo mas grandes en la naturaleza (76.99mm ± 4.23mm) comparado con las de cautiverio (66.58mm ± 2.10mm). Esto muestra que métodos utilizados en cautiverio pueden causar endogamia. Se necesitan técnicas más responsables para la crianza, como la introducción y mantenimiento de la diversidad genética en poblaciones en cautiverio.

INTRODUCTION

Captive breeding, when practiced correctly, can help to maintain fitness and diversity in populations (Balmford 1996), and has been the difference between survival and extinction in many endangered species populations (Derrickson *et al.* 1992, Jones *et al.* 1995, Snyder *et al.* 1996). A unique example of breeding in captivity is in the case of butterfly gardens, as they have different goals in mind. Butterfly gardens use poor rearing techniques to maximize profits, which often result in inbreeding. This is seen by certain gardens breeding over 100 generations of butterflies without the introduction of new genes (Lewis *et al.* 2001). Inbreeding causes a magnification of homozygosis, while also making rare alleles more rare (Barber 2002). Homozygotes tend to have lower fitness than heterozygotes (Barber 2002, Reed *et. al* 2003, Saccheri 1996). In addition, larger population sizes and high genetic variation are correlated with higher fitness (Joron and Brakefield 2003, Reed *et. al* 2003). Inbreeding works with genetic drift to decrease genetic diversity in a population, and usually results in inbreeding depression (Barber 2002). Because butterfly gardens tend to have inbreeding (and therefore greater homozygosis), small population sizes, and low genetic variation, they should also exhibit lower fitness. Evidence of this is seen by an increased extinction rate in inbred populations of fruit flies and mice (Milius 1998).

Inbreeding in captive populations has been found to cause asymmetry and reduced body size in progeny (Clarke 1995, Milius 1998). Fluctuating asymmetry is known as nondirectional variation between left and right sides of the body, or deviations from perfect bilateral symmetry (Breuker *et al.* 2003, Palmer *et al.* 1986, VanValen 1962). Deviations from bilateral symmetry and reduction in antennae and wing size are important determinants of butterfly fitness. Antennae size is critical for pheromone and food sensing as well as flight balance, whereas reductions in wing size are important in terms of flight aerodynamics (DeVries 1987). Increases in fluctuating asymmetry are thought to be negatively correlated with heterozygosis (Gomendio *et al.* 2000), and therefore correlated with inbreeding. It is assumed that identical genes affect bilateral symmetry (Clarke 1995), but asymmetry can also result from developmental stress (Breuker *et al.* 2003).

Lewis *et al.* (2001) found that captive bred populations of the butterfly *Pieris brassicae* had smaller wings and lower wing aspect ratios. However, in addition to inbreeding depression, food quality may also have a negative impact on butterflies (Cassel 1999). This means gardens with poor quality food may have smaller butterflies as a result. However, past studies on *Drosophila* populations have found great reductions in wing size solely due to inbreeding (Robertson and Reeve 1952, Lewis *et al.* 2001, Wright 2007), showing that small wing size can be used as a proxy for inbreeding.

The aim of this study is to compare size reduction in captive bred populations to wild populations in *Morpho peleides limpida*, a large butterfly in Costa Rica (DeVries 1987). Decrease in size will be used as a proxy to measure inbreeding and other developmental stresses associated with butterfly gardens.

METHODS

STUDY SPECIES. ü *Morpho peleides limpida* is a large butterfly common on the Atlantic and Pacific slope of Costa Rica (DeVries 1987). Because it is large, variations in size were greater and easily observed. There is sexual dimorphism; the female is generally larger than the male and has a wider black margin (DeVries 1987). Also, males fly from early morning to midday, whereas females are seen flying only around midday (DeVries 1987). I used these behavioral and morphological differences, along with the fact that the male's claspers can be extruded with gentle pressure to produce a smell like vanilla (DeVries 1987), to sex the butterflies.

STUDY SITE. ü This study was carried out in Monteverde, Costa Rica, from April 26th to May 6th 2009. I captured and measured captive *M. peleides* butterflies at the Monteverde Butterfly Garden, and compared them to wild individuals captured near the Creativa School in Cerro Plano and the San Luis Río Bruja de Monteverde.

Creativa is located at around 1500 m elevation, whereas Río Bruja is around 1100 m elevation. All wild capturing sites were along roadsides near forest fragments. The *M. peleides* butterflies from the Monteverde Butterfly Garden were sampled from Garden One, which is protected from rain, but wind my affect ambient temperature.

MEASUREMENT. ü While collecting data on butterflies in captivity, I avoided resampling by keeping all captive individuals in wax envelopes until all sampling was complete. Wild butterflies were marked with a red paint pen on the dorsal side of the abdomen, to allow future recognition. Right forewing measurements were taken from the distance of the basal area to the tip of the apex, as described by DeVries (1987) (Fig. 1A), and right antennae length was measured from the head to the tip of the antennae (DeVries 1987) (Fig. 1B). All measurements were taken with a caliper in millimeters to the nearest tenth of a millimeter.

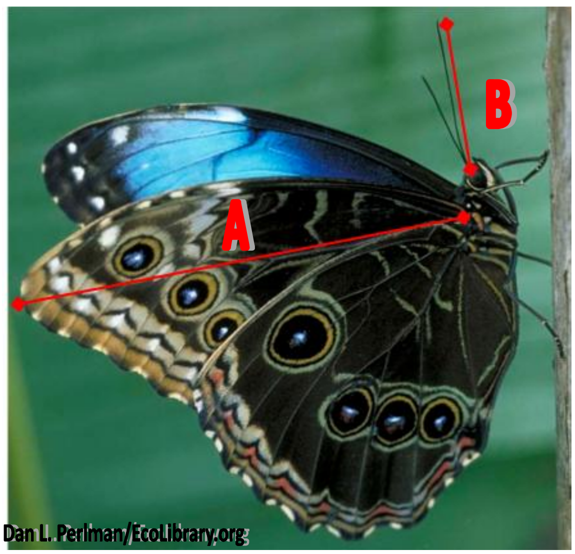

Figure 1: Methods of measuring butterfly forewing (A) and antennae (B), as described by DeVries (1987).

STATISTICAL ANALYSES. ü A two-way MANOVA was used to examine the differences in both right forewing and right antennae between wild and captive bred male and female *M. peleides* butterflies. A MANOVA test is more conservative than two separate two-way ANOVAs, and is more accurate than univariate tests (Avalos *et. al* 2007). I used a two-way ANOVA test to compare right forewing differences in wild and captive populations, and the same procedure was used again for the right antennae. All statistical analyses were done using JMP IN statistical software.

RESULTS

In total, 23 wild and 35 captive butterflies were collected and measured. Captive male populations had a sampling size of 29, and captive female populations had a sampling size of 5. Populations sampled from wild sites had a sampling effort of 13 male and 10 female butterflies. Captive male and female butterflies varied in size from 54.0 to 69.5 mm and 63.5 to 68.6 mm in right forewing length respectively. Wild measurements of male and female sizes varied from 54.8 to 74.7 mm and 71.1 to 82.5 mm in right forewing lengths. Following this trend, right antennae lengths of males were 18.3 to 25.2 mm for captive and 19.6 to 27.1 mm for wild individuals. Females' right antennae ranged in size from 19.7 to 22.5 mm and 22.6 to 27.5 mm for captive and wild populations respectively.

Mean sizes of captive bred populations were shown to be smaller than wild populations in all measurements in both male and female butterflies (Fig. 2). The MANOVA results for the two (right forewing length and right antennae length) indicated significant differences for both sex ($F_{1,53}$ = 44.4503, P < .0001) and treatment type ($F_{1,53}$ = 29.9577, P < .0001). No interaction was found, however, between treatment type and sex ($F_{1,53}$ = 1.2825, P = 0.2625).

A two-way ANOVA indicated significance for right forewing lengths between captive and wild populations ($F_{3,54}$ = 47.1507, P < .0001; Fig. 2a), and between sexes ($F_{3,54}$ = 34.4780, P < .0001; Fig. 2a), with no interaction between the treatment type and sex ($F_{3,54}$ = 2.4615, P = 0.1225; Fig. 2a). A two-way ANOVA for right antennae lengths signified that there was a difference between rearing environments of captive and wild populations ($F_{3,53}$ = 40.4542, P < .0001; Fig. 2b), but no statistical difference is seen between male and female ($F_{3,53}$ = 3.5191, P = 0.0662; Fig. 2b), nor for an interaction between sex and treatment environment ($F_{3,53}$ = 3.2283, P = 0.0781; Fig. 2b).

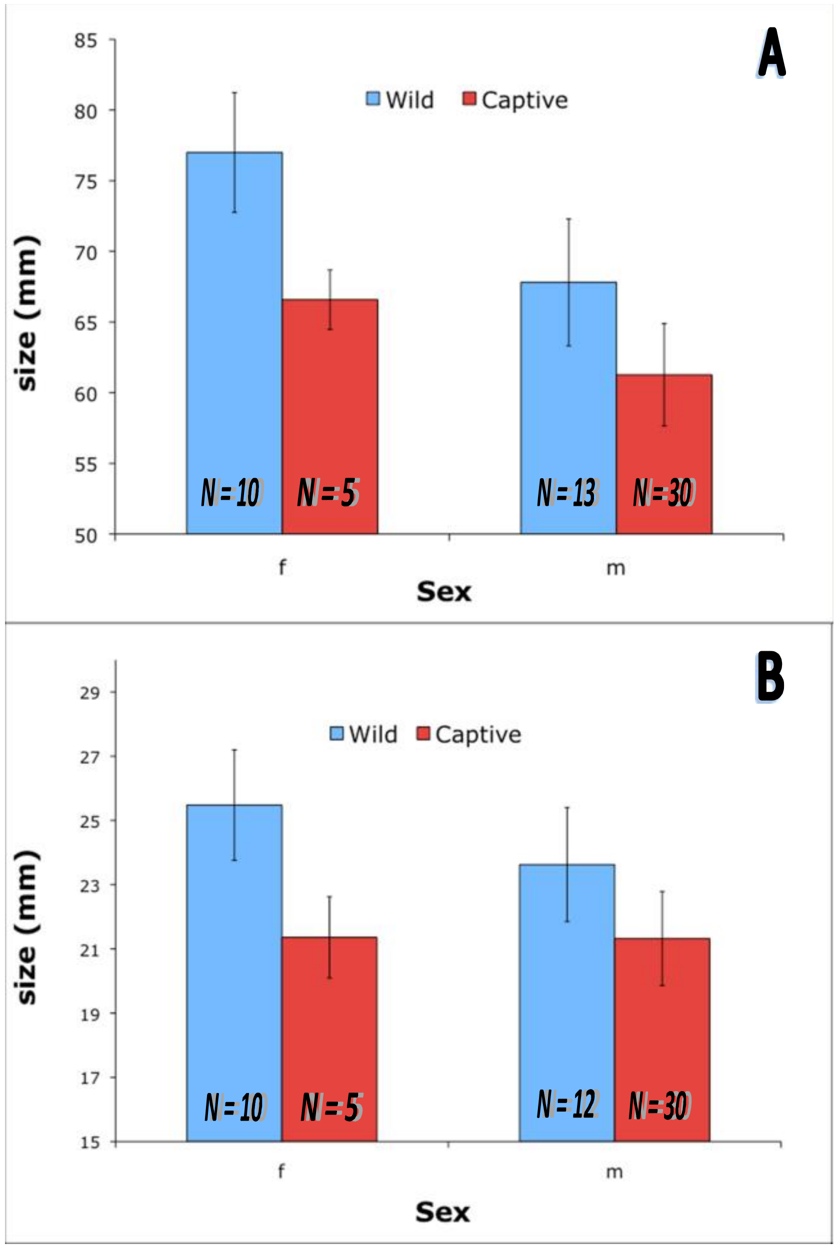

FIG. 2. Mean size (±SE) of right forewing (A) and right antennae (B) of wild and captive populations of *M. peleides*, separated by sex, female (f) and male (m). *N* values are shown in their corresponding boxes.

DISCUSSION

I predicted that wild butterflies would be larger in terms of right forewing and antennae length. Wild butterflies are greater in size than captive butterflies (Fig. 2). During my observations in the Monteverde Butterfly Garden, I witnessed that some *Morpho peleides* butterflies emerged from their cocoons with disfigured wings. The Garden staff informed me this is a clear sign of inbreeding. Since all of these butterflies come from the same breeder, they are all reared together, and likely to have come from the same gene pool. These trends are consistent with the idea that captive breeding (and perhaps therefore inbreeding) reduces body size of individuals.

Because body size can be related to fitness (DeVries 1987), it can be said that inbreeding may reduce the fitness of offspring, consistent with inbreeding depression trends. Inbreeding depression has also been found to act with disease and genetic drift to induce extinction in wild populations (New 1995). This has been previously observed by massive disease outbreaks and bottlenecks during harsh conditions in butterfly gardens (DuPont 2007). Because of these negative effects correlated with inbreeding, captive breeding techniques of butterflies are in great need of improvement.

Some possible steps gardens can take to reduce the impacts of captivity would be to quantify the initial genetic diversity, introduce wild individuals into the population to increase diversity, and make sure all individuals are contributing genetic information to the next generation (Barber 2002). These would all act to reduce the effects of drift and inbreeding depression (Barber 2002). Past experiments have shown that rearing in captivity can select for female butterflies with higher ovary mass and more egg production (Lewis *et al.* 2001). Also, butterflies that reproduce well in captivity may not necessarily reproduce well in the wild (Barber 2002), indicating the importance of retaining all genetic diversity in captive populations.

It has been observed that captive breeding leads to a reduction in size, most likely from inbreeding due to poor rearing techniques. All variables in rearing techniques have not been separated to distinguish how much each factor affects size. Future studies should focus on larval populations with the same degree of inbreeding, and feed them varying qualities of food to see the amount quality of food affects size in captive and wild butterfly populations.

ACKNOWLEDGMENTS

Thanks to all the staff at the Monteverde Butterfly Garden for allowing me to sample their stocks. Thanks to the CIEE students Sonia and Brian for their help in capturing and recording butterflies. Thanks to Pablo Allen for the knowledge of techniques to handle the butterflies and his statistically support. I would also like to thank the Creativa for allowing me to trap and measure their nearby *M. peleides* population.

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