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Variation in the genitalia of the butterfly *Maniola jurtina* (Lepidoptera: Satyrinae)

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The genitalia of animals, particularly insects, are extremely useful taxonomic characters, for they are highly conserved within species yet generally diverge rapidly during speciation: often the only reliable means of separating sibling species is via the morphology of the genitalia. A number of explanations have been proposed to explain this pattern of variation, the most prominent of which is the 'lock-and-key' hypothesis. This hypothesis and others are tested using the meadow brown butterfly, *Maniola jurtina* (L.), which exhibits variation in the male genitalia. A novel technique is described which enables dissection of copulating couples. Sampling and dissection of male butterflies from 14 populations in southern England was carried out to quantify variation in the genitalia. Mating success and the strength of the male-female bond during copulation was assessed in relation to the dimensions of the male genitalia.

The most variable portions of the male genitalia are the distal and dorsal margins of the paired valves. Contrary to the lock-and-key hypothesis, and the more recent sexual selection model, evidence is presented that the most variable portions of the genitalia have no apparent function during copulation, and therefore may not be subject to direct selection. I suggest that neutrality to selection was essential for the evolution of the wide variety in genital morphology currently found in the Lepidoptera.

CONTENTS

INTRODUCTION

The genitalia of male butterflies comprise the highly modified sclerites of the 9th and 10th body segments. Genital morphology, particularly of the male, is one of the principal characters used in most taxonomic studies of the Lepidoptera, and in some studies is the only character used. The conserved nature of intraspecific variation was for many years attributed to a 'lock-and-key' fit between male and female genitalia, which served to prevent trans-specific mating (Rentz 1972). There is disturbingly little evidence to support this hypothesis. The work of Lorkovic (1953, 1957) principally on satyrine butterflies, describes a considerable degree of variation within species. He also

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examined the relative positions of the male and female genitalia during copulation and states that there is considerable potential for variation in the shape of the male genitalia which would not affect copulatory success. This is supported by successful inter-race crosses in the butterfly, *Papilio dardanus* where the subspecies differ widely in valve shape (Carpenter, 1948; Clarke & Sheppard, 1960a, b; Turner, Clarke & Sheppard, 1961). Another criticism of the lock-and-key hypothesis is that it does not explain the trend towards greater genital complexity in the male than the female, found across all animal phyla (Eberhard, 1985).

A number of other hypotheses have been proposed as alternatives to the lock-and-key (Eberhard, 1985 provides a full discussion of each). The most plausible are briefly described here. Mayr (1963) suggested that changes in genital morphology during speciation are the result of pleiotropic effects of many genes, each subject to selection via their other effects upon phenotype. A necessary condition of this argument is that genitalia are not constrained by a lock-and-key fit.

Lastly, Eberhard (1985) proposed the "sexual selection by female choice" hypothesis. He argues that genital structure of the male plays an important role in courtship, via stimulation of the female during copulation, and as such is subject to runaway selection (Fisher, 1958). As Turner (1962) pointed out, confirmation or rejection of hypotheses regarding the evolution of genital morphology can only come from studies of the physiology of copulation. This study attempts precisely that, using the butterfly, *Maniola jurtina* (L.).

Maniola jurtina exhibits a considerable degree of genital variation, both within and between populations, particularly with regard to the shape of the paired valves (Thomson, 1973). This has led to a profusion of taxonomic work: 113 names have been used to describe taxonomic subdivisions of *M. jurtina* (Thomson, 1973). This unsatisfactory situation remains despite attempts to clarify matters by Thomson (1973, 1975) and Higgins (1975).

MATERIALS AND METHODS

To quantify the extent of variation within and between populations of *M. jurtina* in southern England 1086 male butterflies were captured from 14 sites (at least 25 per site) during 1989 and 1990, and the genitalia dissected.

Dissection procedure

The tip of the abdomen was first softened by soaking overnight in 10% NaOH. The genitalia were teased from the surrounding tissue and soaked overnight in absolute alcohol to dehydrate, and thus render transparent any remaining tissue. The genitalia were then mounted in Euparal under a cover slip on a numbered slide. The maximum and minimum width and length of the valves were recorded (Fig. 1). These measurements were used in preference to Thomson's (1973) *F* value, as this was found to be subjective, and entirely inapplicable to certain rare valve shapes. Measurement of maximum and minimum widths was carried out to give some indication of the height of the dorsal process, one of the most variable aspects of valve shape.

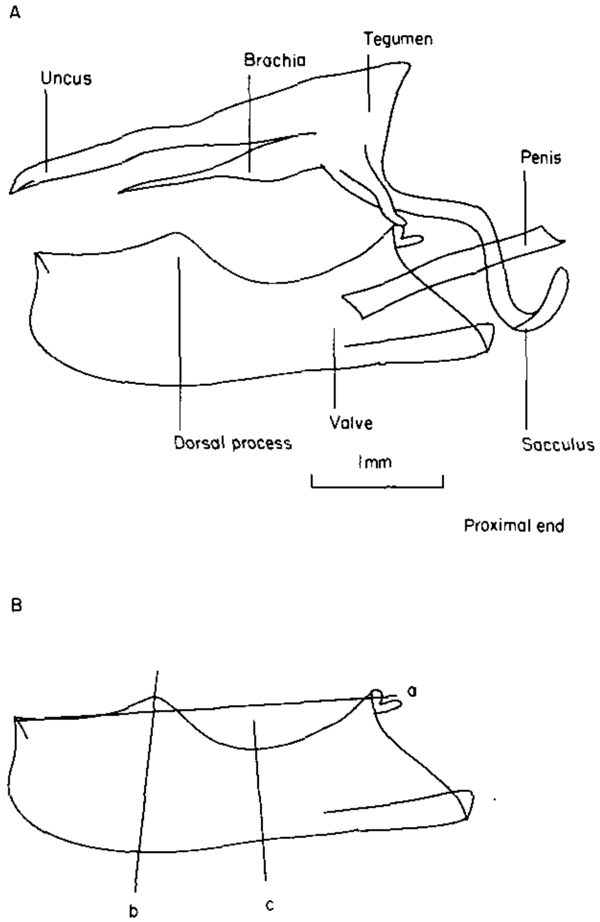

Figure 1. The male genitalia of *M. jurtina*. A, Lateral view of genitalia; B, valve showing dimensions measured; a = value length, b = value width (max.), c = value width (min.).

To assess the significance of variation in the male genitalia 12 copulating pairs were dissected. Unfortunately copulating pairs of butterflies separate easily when disturbed, preventing examination of their relative positions under a dissecting microscope. Gentle cooling in a refrigerator in an attempt to render them less active proved useless as this provokes immediate separation. Sudden immersion in liquid nitrogen was the only technique which killed the butterflies instantaneously, and allowed dissection of the genitalia in copula.

To evaluate whether the shape of the male genitalia influences mating success 32 copulating males were captured and the dimensions of their genitalia compared to the population mean.

To assess the strength of the male-female bond during copulation in relation to valve shape, 14 pairs found in copula were frozen in liquid nitrogen as above, and then thawed to room temperature. A paper clip was used to grip the closed wings of each butterfly, and the couple suspended by the clip attached to the female. Half gramme weights were then hooked through the clip attached to the male until their weight pulled the butterflies apart. The number of weights used was recorded.

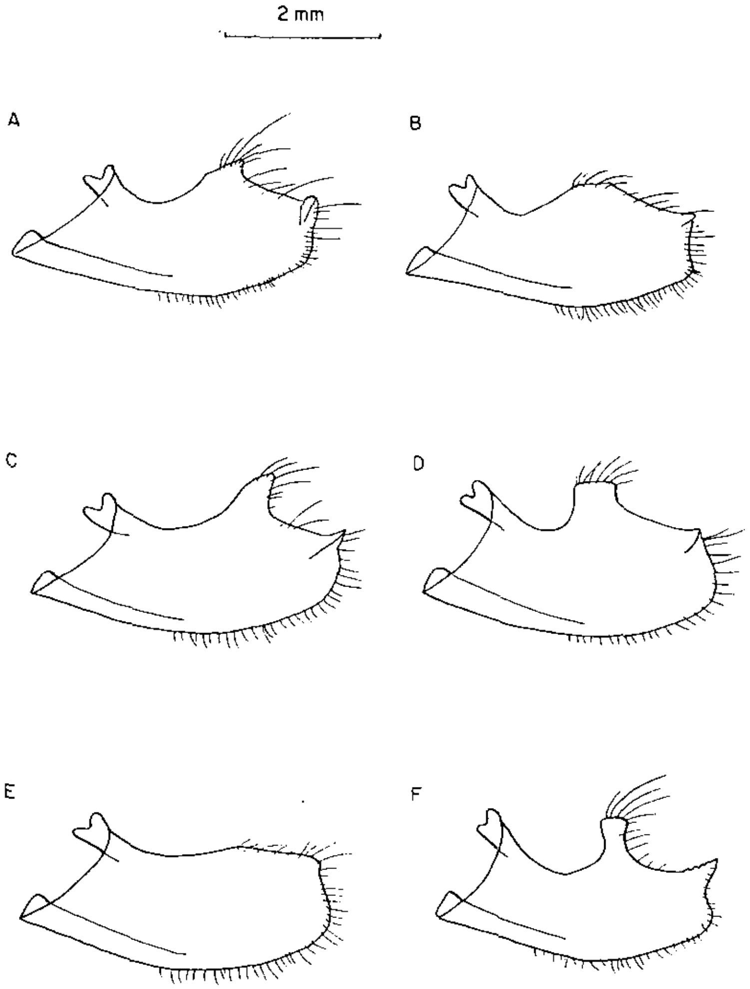

Figure 2. Variation in valve shape: six valves sampled from Bernwood Meadows, 1989.

RESULTS

The shape and size of valves was extremely variable within all populations sampled: Fig. 2 illustrates six valves taken from one population, Bernwood Meadows. The most variable portions of the valves were the dorsal and distal margins, particularly the size of the dorsal process. The dorsal process varied continuously in size: at its largest it was 0.61 mm tall, while in some specimens it was entirely absent. No significant differences were found between populations in the height of this process (one-way analysis of variance, $P = 0.351$) (full data are presented in Goulson, 1992). Valve length also varied considerably, from 1.51 to 2.35 mm ($\bar{x} = 2.09$ $\sigma = 0.29$, sites combined). This was largely a function of butterfly size, for the Pearson correlation coefficient between wing length and valve length was 0.886 ($N = 1086$).

Dorsal and lateral views of the genitalia during copulation are shown in Fig. 3. The surfaces in contact with the female are the ventral edges of the

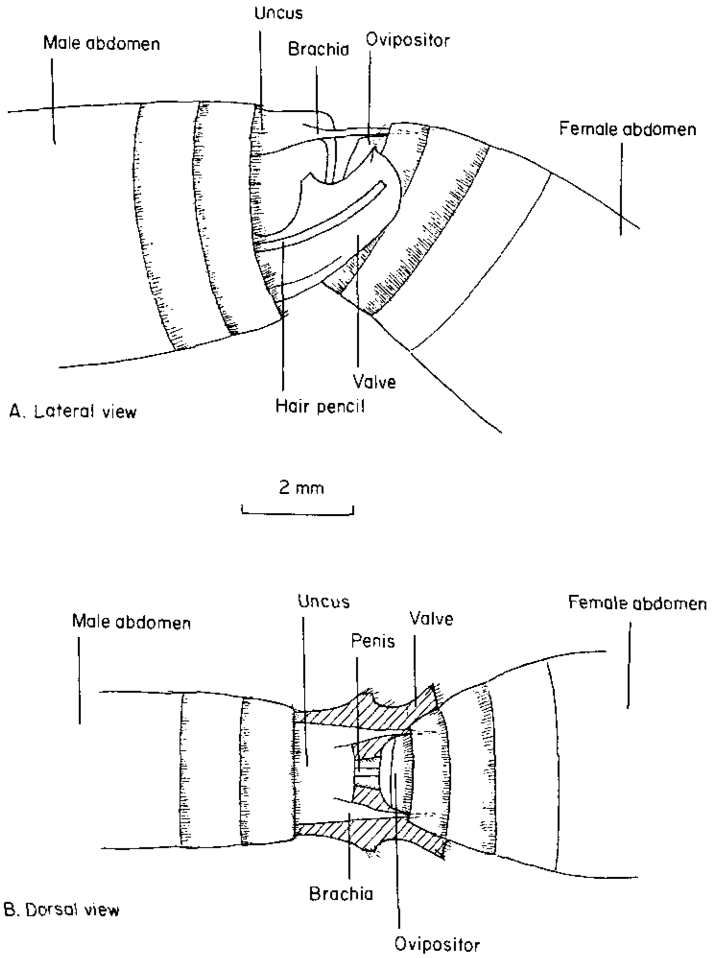

Figure 3. Genitalia during copulation. A, Lateral view; B, dorsal view.

valves, which grip the female ovipositor, and the paired brachia which pass between the ovipositor and the tergite of the 8th abdominal segment. Therefore, the most variable parts of the valve, the dorsal and distal margins do not have any obvious function. The male-female bond does not depend on precise matching of morphological structure.

The dimensions of the valves of mated butterflies did not differ significantly from that of non-mated butterflies (valve length, $\bar{x} = 2.10$ mm $\sigma = 0.31$, $\bar{x} = 2.09$ mm $\sigma = 0.28$, dorsal process, $\bar{x} = 0.14$ $\sigma = 0.11$, $\bar{x} = 0.10$ $\sigma = 0.05$, mated and non-mated butterflies, respectively). This suggests that the dimensions of the valve do not affect mating success, for probability suggests that butterflies caught while copulating should on average mate more times than those which were not copulating when caught.

No relationship was found between valve dimensions and the weight required to separate copulating couples (Pearson correlation coefficients 0.131, -0.011 for valve length and dorsal process respectively).

DISCUSSION

This study shows that, at least in *M. jurtina*, the male and female genitalia do not form a precise lock-and-key. The portion of the genitalia used to classify races of *M. jurtina* in Europe (Thomson, 1973; Shreeve, 1989), namely the dorsal process, has no obvious function during copulation. This suggests that variation may be confined to non-functional parts of the valve, and is in accordance with Lorkovic's view that male genital morphology is not tightly constrained: a degree of variation may not be subject to direct selection. This is supported by the absence of any apparent relationship between valve shape and either mating success or strength of the male-female bond.

If variation in the shape of a large portion of the valve is not constrained by selection, then differences between races or subspecies in the valve shape are unlikely to act as barriers to cross-fertilization. Clearly there is some doubt as to the accuracy of the lock-and-key analogy. This is of some concern to taxonomic studies in which the genitalia are assumed to provide one of the most reliable characters by which species may be distinguished. The use of genitalia in such studies must be reconsidered: if intraspecific variation in valve shape is not tightly constrained by selection then how reliable is its use in describing species? The consistent use of valves in taxonomy over many years may be reason enough to accept their use as valid, for if it regularly produced anomalies and inconsistencies they would long ago have been discarded as an important tool. Why then does the valve shape of the Lepidoptera remain a valuable taxonomic character? I would argue that evolution of male genital morphology is best considered in two parts, those which contact the female during copulation, and those which do not. The sexual selection of female choice model and the lock-and-key hypothesis are both only of relevance to those portions of the genitalia in contact with the female. The rest of the genitalia may be neutral to selection and subject only to random processes, or to pleiotropic effects as suggested by Mayr (1963).

This study may explain why genitalia are so valuable in taxonomic studies, for neutrality to selection is one of the key features used by the traditional school of evolutionary taxonomy to select characters for use in taxonomic studies, for analogies (as opposed to homologies) are less likely to evolve when selection is absent (Ridley, 1986).

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REFERENCES

Carpenter GDH. 1948. Notes on the males of *Papilio dardanus* Brown (Lep.) with the definition of a new transitional race and a redescription of *P. dardanus ochracea* Poulton. *Proceedings of the Royal Entomological Society of London (B)* **17:** 11–17.

Clarke CA, Sheppard PM. 1960a. The genetics of *Papilio dardanus* Brown II. Races *dardanus*, *polytrophus*, *meseres* and *tibullus*. *Genetics* **45:** 439–457.

Clarke CA, Sheppard PM. 1960b. The genetics of *Papilio dardanus* III. Race *antinorii* from Abyssinia and race *meriones* from Madagascar. *Genetics* **45:** 683–698.

Eberhard E. 1985. *Sexual selection and animal genitalia*. Cambridge, Massachusetts: Harvard University Press.

Fisher R. 1958. *The genetic theory of natural selection*. New York: Dover.

Goulson D. 1991 *Maintenance of phenotypic variation in the butterfly*, Maniola jurtina. Unpublished PhD thesis, Oxford Polytechnic.

Higgins LG. 1975. *Maniola jurtina* and its subspecies. *Proceedings of the Royal Entomological Society of London (C)* **34:** 1–2.

Lorkovic Z. 1953. L'accouplement artificiel chez les Lepidoptères et son application dans les recherches sur la fonction de l'appareil genital des insectes. *Physiologia comparata et oecologia* **3:** 313–320.

Lorkovic Z. 1957. Die Speciationsstuffen in der *Erebia tyndarus*-Gruppe. *Biološki glasnik* **10:** 61–110.

Mayr, E. 1963. *Animal species and evolution*. Cambridge, Massachusetts: Harvard University Press.

Rentz D. 1972. The lock and key as an isolating mechanism in katydids. *American Scientist* **60:** 750–755.

Ridley M. 1986. *Evolution and classification, the reformation of cladism*. London: Longman.

Shreeve TG. 1989. The extended flight period of *Maniola jurtina* (Lepidoptera: Satyridae) on chalk downland: seasonal changes of the adult phenotype and evidence for a population of mixed origins. *The Entomologist* **108:** 202–215.

Thomson G. 1973. Geographical variation of *Maniola jurtina* (L.) (Lepidoptera: Satyridae). *Tijdschrift voor Entomologie* **116:** 185–226.

Thomson G. 1975. Les races de *Maniola jurtina* (L.) en France et pays voisins. *Alexenor* **9:** 23–32; 53–66.

Turner JRG. 1962. A method for mass producing mounts of lepidopteran genitalia, with a note on their evolutionary significance. *The Entomologist* **95:** 146–148.

Turner JRG, Clarke CA, Sheppard PM. 1961. Genetics of a difference in the male genitalia of East and West African stocks of *Papilio dardanus* (Lep.). *Nature* **19:** 935–936.