ESPECIALLY FOR FIELD COLLECTORS

(Under the supervision of JAMES R. MERRITT)

HAND-PAIRING OF BUTTERFLIES

by C. A. CLARKE and P. M. SHEPPARD

The ability to effect copulation in certain species of butterflies by the method of hand-pairing has been known for many years. In England early in this century a well known professional dealer used the method to mate *Papilio machaon* but it remained a secret "held so tightly that only rumours reached the ears of his friends" (T. W. JEFERSON, 1952, personal communication). In 1919 SWYNNERTON, working with *Papilio dardanus* in Africa, gave details of the method which he used to obtain artificial matings:- "Pairing now offers little difficulty. I have made it compulsory. The genitalia are brought into the correct juxtaposition, very slight pressure is exercised on the sides of the two abdomens with finger and thumb of each hand, and as soon as the male is seen (by abdominal movements) to take on, he is allowed to hang and both are placed in a box, better dark. Some males refuse".

FORD (1936) drew attention to the method when writing of the genetics of *Papilio dardanus* and thought that it was so important that it should be used in all future work on this butterfly. He pointed out that results based on a study of females fertilised in nature are always open to suspicion because of the possibility that broods may be of mixed paternity. SWYNNERTON'S method obviates this and moreover, if it is possible to mate the same male with two or more females of different forms (such as are found in *dardanus*) the genetic analysis is greatly simplified. Hand-pairing is also referred to by FORD (1945) in *Butterflies,* where he mentions that the wings of the female should be held either with a clip of the type used on retort stands or simply in a cleft cane.

LORKOVIC (1947, 1953, 1954) pointed out that natural pairing of butterflies is an instinctive act composed of several successive reflexes initiated by sight, odour, and touch and influenced also by external conditions such as temperature. It is also dependent on the integrity of the nervous system. In captivity butterflies are often unwilling to pair because the precise conditions necessary are not easy to obtain artificially; as a result matings in many species are effected only with the greatest difficulty or not at all. It is well known, however, that in some insects such as the mantis, copulation can be carried out successfully even when the abdomen is severed from the body. LORKOVIC, therefore, thought that in butterflies ablation of the head and thorax might enable pairing to take place more readily because inhibitory influences initiated by the cerebral ganglia might then be removed. On testing this hypothesis he found that separation of the abdomen from the head or, more simply, that squeezing the head and thorax of the male (so as to render the insect semi-paralysed) enabled matings to be obtained in many species of butterflies. The details of his method are as follows:- The female butterfly is placed on a table and the abdomen is pulled

out from between the wings and a pin put in above it. The wings are folded above the body and are kept in place by a piece of glass. (See fig. 1.) The abdomen of a male butterfly, emerged preferably two or three days previously, and in whom the head and thorax have been crushed, is then taken in forceps and placed against the copulatory organs of the female. With a second pair of fine forceps the claspers of the male are opened and the two abdomens brought into apposition: at this moment the uncus engages, the claspers close round the body of the female, and thrusting movements by the male indicate that pairing has begun. When this occurs the female is removed from under the glass and placed on some object so that the male can hang from her abdomen. When pairing is finished the male breaks away from the female and falls down. The insect generally recovers and can then be used for subsequent matings.

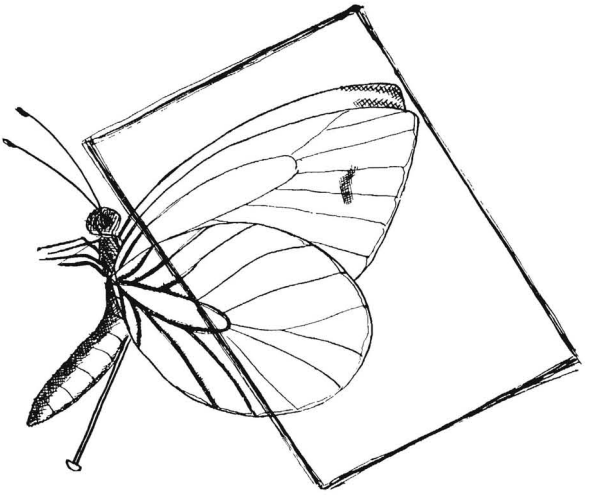

Fig. 1. Lorković method of positioning a female.

The method has been particularly useful in the Papilionidæ and Pieridæ, and LORKOVIC states that he has had no failures in any of the European members of these families which he has tested, except in the genus *Leptidea.* He has also succeeded in the Satyridæ with many species of *Erebia, Pararge,* and *Cœnonympha* and among Nymphalidæ with the genera *Limenitis, Neptis,* and *Melitæa.* Success is also claimed with some of the Hesperiidæ. In Lycænidæ the method is difficult to apply because in this family the genital armature is buried very deeply in the abdomen and it only emerges by reflexes provoked by the excitement of sight or scent.

The following list shows the various interspecific crosses which LORKOVIC has obtained, and he considers that method is here all-important because behavioural barriers would normally be a bar to copulation in these butterflies. It also enables successive broods to be obtained even when external factors are adverse — for instance it is invaluable for the continued breeding of *Colias croceus* through out the winter.

1. *Papilio p. podalirius* × *P. p. feisthamelii*
2. *Pieris rapæ* × *P. manni* (hybr. *lorkovici*)
3. *Pieris napi* ♂ × *P. ergane* ♀ (hybr. *naperga*)
4. *Pieris napi* ♂ × *P. manni* ♀ (hybr. *namanni*)
5. *Pieris napi* ♂ × *P. rapæ* ♀ (hybr. *narapæ*)
6. *Pieris rapæ* ♂ × *P. daplidice* ♀
7. *Pieris napi* ♂ × *P. daplidice* ♀
8. *Euchloe cardamines* ♂ × *E. euphenoides* ♀ (hybr. *cardaphenoides*)
9. *Euchloe cardamines* ♂ × *E. belia* ♀ (hybr. *cardabelia*)
10. *Erebia stirius* ♂ × *E. pronoe* ♀
11. *Erebia æthiops* ♂ × *E. styx* ♀
12. *Erebia tyndarus* ♂ × *E. ottomana* ♀
13. *Hesperia alveus* ♂ × *H. armoricanus* ♀

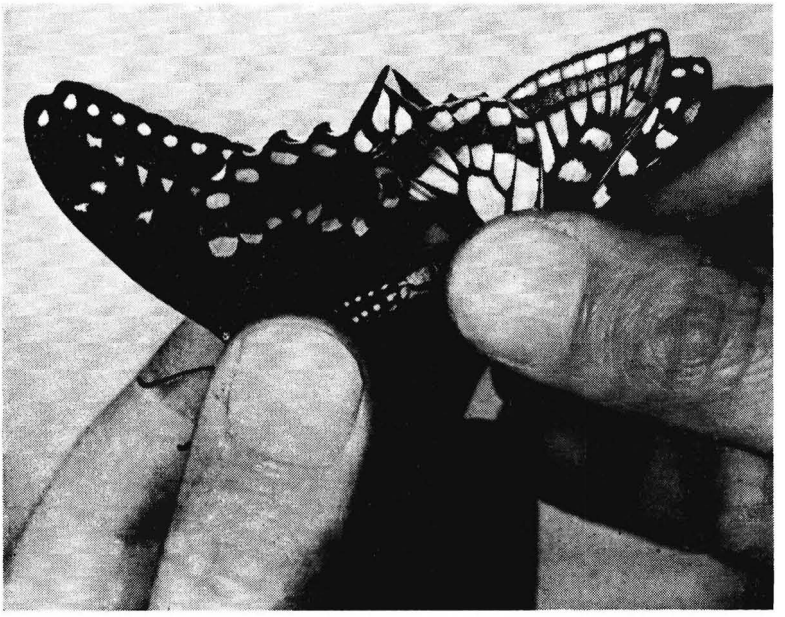

Fig. 2. Technique of hand-pairing; ♀ *P. asterius* and ♂ *P. machaon.*

CLARKE (1952), working independently with *Papilio machaon,* found that it was easy to hand-pair this butterfly and that much less attention to detail was necessary than with "natural" matings obtained under greenhouse conditions. The method he employed was essentially that of SWYNNERTON, the details being as follows:-First the butterflies are warmed in a cage for about ¼ hour at a temperature of between 65° and 80° F. They are then held as shown in figure 2, the male being easily distinguished by the triangular claspers on the last abdominal segment. Slight pressure causes the male to open his claspers widely and these then embrace the terminal segment of the female, correct positioning being facilitated by slight rotatory movements on the part

of the operator. After some minutes "locking" occurs and moderate tension on the abdomens does not then cause separation. Simultaneously the head and thorax of the male appear to become lifeless. The butterflies normally remain together for half to three quarters of an hour, during which time thrusting movements by the male indicate that insemination is taking place. The photographs (fig. 3) show a pair of butterflies after hand-mating.

Fig. 3. *P. dardanus cenea*: ♀ form "leighi" pairing with ♂.

Over 1,000 matings have been carried out in this way during the past three years and the method has been successful with the following species and subspecies:- *P. machaon* (various races), *P. hospiton, P. machaon hippocrates, P. podalirius, P. demodocus, P. xuthus, P. asterius, P. bairdii, P. podalirius, P. zelicaon, P. indra, P. glaucus, P. rutulus, P. thoas, P. dardanus, P. polytes.*

It should be pointed out that LORKOVIC'S method of severing the abdomen has not been employed by us. Futhermore, on the occasions when our technique has failed, compression of the head and thorax has not brought about successful pairings and in our experience males maimed in this way do not survive so well for future matings, although they probably would do so in warningly coloured species which have tougher bodies.

Certain difficulties in our technique of hand-mating have been found in all the forms tested, and the following observations on this matter may be helpful.

(1) There is considerable variability with regard to the ease with which mating can be effected. Some males refuse all females, and many refuse some, though they may mate with a previously refused female after an interval of some hours, or even a day or two. The same remarks apply to females. If pairing does not occur within a very few minutes the butterflies should be left for a few hours.

(2) In the *machaon* group males of the races *britannicus, gorganus,* and *mediterraneus* appear to copulate much more readily than do those of *asterius, brevicauda,* and *zelicaon.* This is so marked that it is usually easier to obtain hybrids with these three butterflies using *machaon* males than it is to effect matings using males of their own species.

(3) Care must be taken to see that the butterflies are correctly approximated. Sometimes they appear to be so, but closer inspection with a hand lens may show the ædeagus to be improperly engaged. This is particularly so in *dardanus,* and in this species the bodies should be at an angle of less than the usual 180°. Matings lasting less than 10 minutes or more than 3 hours, particularly the former, may be unsatisfactory.

(4) Some of the insects of all the species tested have soft, flabby bodies and these, particularly the male, are most difficult to hand-pair. In *P. perrhebus* successful matings have never been obtained because of flabbiness and inability of the male to "take on".

(5) Some males appear willing but the ædeagus retracts when brought into contact with the female. Such insects are often unsatisfactory but successful pairing sometimes takes place after an interval.

LAYING

After a period of time, varying between one and seven days, the females may start laying, a temperature between 70° and 90° F. being optimum. The habits of the different species vary. (1) The *machaon* group oviposit best when allowed to flutter unconfined on the growing food plant (fennel or rue) in the greenhouse. A minority, however, will lay in cages to picked food plant. (2) *P. glaucus* and *P. rutulus* need to be walked repeatedly up a flat leaf of *Liriodendron.* (3) In *P. dardanus* it seems obligatory for the butterfly to be sleeved on the growing food plant. These insects flutter like moths before depositing and frequently lay best in the evening under a strong electric light.

In all the species tested there is very great variability in the number and fertility of the eggs laid; 70 eggs is a good total but some females produce more. Not infrequently even after apparently satisfactory pairings either no

eggs or infertile ones are produced. Length of life in the butterflies is also variable, with females living from a few days up to three weeks. Life can be prolonged by keeping the insects cool when they are not in use, and by feeding daily in hot weather with sugar and water. This, in our opinion, is preferable to honey and water which may make the legs of the insects sticky and more liable to be torn off during the laying period.

APPLICATION OF HAND-PAIRING TO THE STUDY OF THE GENETICS OF THE PAPILIONIDÆ

In the *machaon* complex it was found that many of the North American, European, North African, and Japanese subspecies could be freely hybridised, and those that have been obtained are shown in the following list. Nearly always the F_1 of these pairings were infertile *inter se,* yet offspring could often be obtained by backcrossing the hybrids to either parent form. This has enabled some information to be obtained as to the method of inheritance of various characters. (Clarke & Knudsen, 1953, and Clarke & Sheppard, 1953, 1955a, 1955b, 1955c, and in press.)

1. *P. asterius* ♀ × *P. machaon* ♂ and reciprocal cross
2. *P. asterius* ♀ × *P. brevicauda* ♂ and reciprocal cross
3. *P. zelicaon* ♀ × *P. machaon* ♂ and reciprocal cross
4. *P. asterius* ♀ × *P. zelicaon* ♂ and reciprocal cross
5. *P. brevicauda* ♀ × *P. machaon* ♂
6. *P. brevicauda* ♀ × *P. zelicaon* ♂
7. *P. machaon* ♀ × *P. bairdii* ♂
8. *P. machaon* ♀ × *P. indra* ♂
9. *P. machaon* ♀ × *P. hospiton* ♂
10. *P. machaon hippocrates* ♀ × *P. machaon* ♂ and reciprocal cross
11. *P. asterius* ♀ × *P. machaon hippocrates* ♂

In *P. glaucus* information is accumulating as to the method of inheritance of the black and yellow female forms, the mating of the same male to the two different kinds of females having been effected on several occasions. *P. glaucus* has also been crossed with *P. rutulus* and a fertile backcross to *P. glaucus* has been obtained. (Clarke & Sheppard, 1955b.)

A start has also been made on breeding *P. dardanus cenea* in England. Again it has been found possible to mate the same male with more than one type of female. This has enabled some preliminary work to be done on the genetic difference between the the female forms "leighi" and "hippocoonides". The method is of particular importance when the characters concerned are sex-controlled to the female, as so often in mimicry.

It is clear that the application of the technique of hand-pairing to the Papilionidæ has shown that they provide some of the most suitable material ever investigated in animals for studying the process of speciation in detail. Moreover the method can be used with great advantage to study the genetics of the polymorphism and mimicry which occur so frequently in this family.

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ARCTIC COLLECTING IN NEW ENGLAND

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When the southern edge of the last great ice sheet had been forced to recede northward and had reached the latitude of northern New England there appeared mountains of imposing height, the White Mountains of the present day. Because of their loftiness they extended up into layers of air so cold that