

Chapter 2

Historical Perspective

Keywords Accessory glands • Aedeagus • Territory • Age • Bursa copulatrix • Copula duration • Ductus ejaculatorius • Eupyrene sperm bundle • Hill-topping • Patrolling • Spermatophore

Male butterflies move over a large area in search of receptive females, infrequently returning to the same area, or patrol within a small area, frequently returning to the same locality. Factors affecting mate-locating behaviour have been mentioned traditionally in the abiotic environment, such as ambient temperature, as well as the biotic environment, such as body temperatures, lek assembly, and territorial perching. Visual cues can be used in the detection and discrimination of conspecific individuals by both sexes, because of their diurnal activities, although some species detect females by scent alone. Various courtship displays have been described in ethology. After adequate courtship behaviour by males, virgin females of most species will accept copulation. Until 30 years ago, it was believed that females in the majority of species mate only once, but that males have the capacity to mate more than once. A plug secreted by males of some species during copulation seemed to prevent further mating of females, supporting such female monogamy. Mate refusal postures of mated females when encountered by males also suggested maintaining female monogamy. Consequently, virgin females are receptive, showing little choice to mate, and mated females are faithful. However, counting the number of spermatophores in the bursa copulatrix of females has indicated that females of most species regularly mate more than once, because a single spermatophore is transferred from the male during a single copulation. Further matings occur after sperm reserves or nutrients are depleted, suggesting that females are not continuously available to mate. Although the sex ratio is unity, for most of the time there are more males than receptive females, and in such circumstances males are in competition to gain access to scarce receptive females. Therefore, female mate choice, particularly in mated females, would be favoured.

Protandry

Mate location, courtship and copulation are the typical three stages of the mating process in butterflies. Both sexes have to find each other; then, the female must evaluate whether the male is suitable for her mate. If the female accepts, copulation and insemination might ensue, after which the female would start laying eggs.

Populations of temperate insects typically have discrete generations of reproductive adults once or a few times during the favourable seasons for the activities, depending on the number of generations they have in a year. The seasonal synchronisation of adult emergence causes most temperate insects to be protandrous, which means that males on average enter the reproductive population some time before the females. There are many examples in the literature showing that male insects are apt to contribute to the maintenance of protandry through rapid development during their larval stage (Forsberg and Wiklund 1988). Theories on protandry have been developed in terms of sexual selection acting either on males to maximise the expected number of matings (Iwasa et al. 1983) or on females to minimise the pre-reproductive period (Bulmer 1983). Therefore, male mating opportunity must be maximised by emerging before females, with late-emerging males being heavily penalised in evolution because of the small number of receptive females to be encountered. Although males emerging too early risk premature death before any receptive females have emerged, most males could have the chance of encountering a large number of receptive females when they adequately start to eclose. Therefore, the optimum time lag in the emergence time between sexes should be apparent.

Various mathematical models on protandry have been developed with the one key assumption that females mate only once (Fig. 2.1). Wiklund and Fagerstrom (1977) advanced the hypothesis that protandry is the optimal reproductive strategy of males, particularly in species maintaining female monandry. The protandry

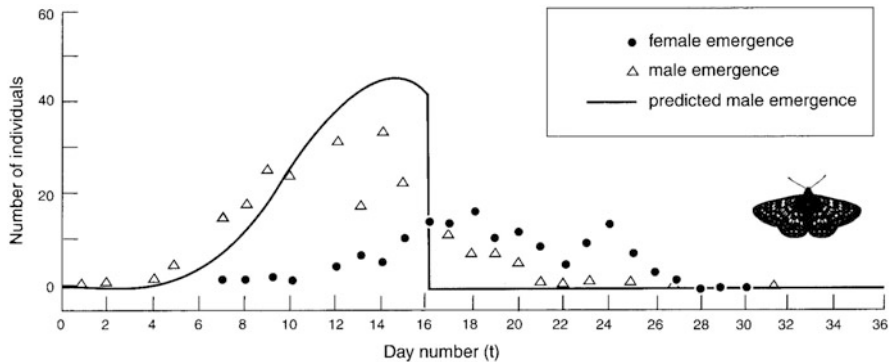
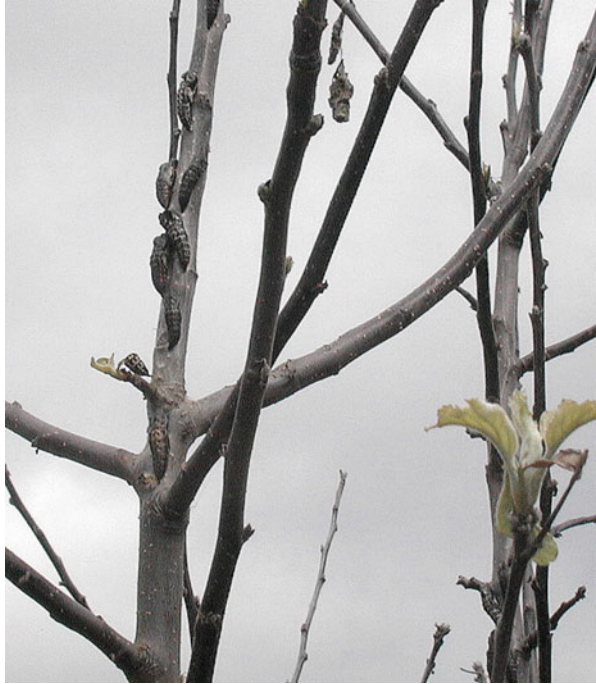


Fig. 2.1 Emergence curves of the checkerspot butterfly, *Euphydryas editha bayensis*, predicted for males using the model of Iwasa et al. (1983) and observed for both sexes. (From Boggs and Nieminen 2004)

Fig. 2.2 Pupae of the black-veined white, *Aporia crataegi*, on a twig of the host tree, *Prunus ssiori*



theory also focusses on species in which the generations are completely discrete (Singer 1982). As might be expected, because females are polyandrous in nature, protandry in respect to emergence is found in only a few species, in which males aggregate around female pupae that are close to emergence to mate with them. Watanabe (1978) observed that males of the black-veined white, *Aporia crataegi*, visited the pupae on the twig of the larval host tree, *Prunus ssiori*, to find females now eclosing (Fig. 2.2). Deinert et al. (1994) also reported that males of the Hewitson's long wing, *Heliconius hewitsoni*, search the larval host plants for female pupae, inspect these pupae regularly, then compete with each other for a position on a pupa and for access to the female as she ecloses. They could find female pupae not by adult appearance but by odour cues, as in the case of the common grass yellow, *Eurema hecabe* (Takanashi et al. 2001). For perching and guarding pupae with periodically assessing the developmental stage in *Heliconius* butterflies, Estrada and Gilbert (2010) pointed out that learning pupal location is a key factor in the mating strategy of the males.

Because selection for protandry is the strongest in species where females are monogamous (Fagerstrom and Wiklund 1982), it might be less important in species with an increased degree of female polyandry. Males of the monandrous peacock butterfly, *Inachis io*, set up and defend territories along forest edges several days before the arrival of females, after hibernation (Wiklund et al. 2003). On the other hand, multivoltine butterflies generally have overlapping generations, particularly

towards late flying season. Considering that polyandrous females have no restraints placed upon them by males against subsequent matings, it can be assumed that there is no strong selection for protandry in these species. Zonneveld (1992) showed that with increasing degree of polyandry, protandry decreases, a result which is independent of any specific parameters. Wiklund and Forsberg (1991) pointed out that the absence of protandry in polyandrous species must be explained by sperm competition.

Encounter

Flight is essential to butterflies in looking for mates, food, and new habitats, to escape from predators, and, in females, for locating oviposition sites. These activities in turn affect the population structure and the operational sex ratio. The efficiency of flight and the capacity of butterflies to sustain flight activity is related to the initial thoracic temperature on takeoff and rates of heat production and loss. Watanabe and Imoto (2003) mentioned that the pale clouded yellow, *Colias erate*, inhabiting open grasslands, maintains a steady thoracic temperature during flight, despite the increasing heat from muscle contraction and radiation, and that air flow over the body (i.e., forced convection) effectively dissipates heat produced by muscle metabolism and radiation in the butterfly body. They show sustained and vigorous flight to be necessary for male mating success (Watanabe et al. 1997). It is thus important for survival in the pale clouded yellow that voluntary flight in open habitats results in the dissipation of excess heat.

To find a mate, male butterflies either fly in search of flying or alighted females or settle in particular localities in wait for flying females. For the former males, the flying behaviour might involve moving along the edge of hedges, woodland margins or forest rides, probably resulting in finding a mate. In some species, the males aggregate in special areas where some interaction within the species might be occurring. For the latter males, they usually occupy and defend the specific area such as forest gaps or sunflecks in the forest floor to increase the frequency with which they encounter receptive females, although there seem to be no obvious resources. Bergman and Wiklund (2009) mentioned three possible reasons why males in temperate forest-dwelling butterflies choose sunflecks or gaps as their perching sites, probably engaging territorial behaviour.

1. If such sunlit areas represent suitable rendezvous sites for males to encounter receptive females, those females must have a preference for visiting sunlit sites.
2. Because the sunlit areas allow males to keep a high body temperature, this may be beneficial for flight performance and the ability to take off rapidly and intercept passing receptive females on the wing.
3. Because sunlit areas are brighter than the surrounding forests, this might facilitate visual detection of receptive females when flying through the sunlit areas.

Within a territory, males of some species often perform conspicuous aerial interactions (Kemp and Wiklund 2001). Although the territoriality is influenced by weather, habitat type, vegetation structure, density of the local population and learning of the adults in the speckled wood butterfly, *Pararge aegeria* (Shreeve 1987), territorial perching males generally have higher mating probabilities than patrolling males (Vande Velde et al. 2011). Males exhibit display functioning as a preliminary fight against the intruders. The two males fly around each other followed by a chase without apparent physical attacks. Although bigger males are more successful in defending and overtaking territories (Rosenberg and Enquist 1991), the winner is usually the resident male. When a female flies into the territory, the resident male could detect her visually and chases her to copulate (Rutowski 1991). When the female flying into the territory alights nearby, the resident male also alights, walks to her and copulates (Wickman and Wiklund 1983).

Hill-topping behaviour is also an effective way of getting the sexes together (Vane-Wright 2003). Other features in the landscape could act as focal points, such as tall trees and gaps in a forest, both of which are occupied by males of some species that perch and wait for receptive females to come along, dashing out to investigate anything that moves. Hill-topping males defend perches on plants from which vantage points they can presumably detect the incoming females. While waiting for females, males perch for variable periods during the daytime on exposed branches and twigs of shrubs, as described by Alcock (2010). Therefore, various components of the hill-topping behaviour, such as perch site selection and site fidelity, as well as the contest behaviour, have to be considered separately in terms of their adaptive significance.

An alternative male tactic is patrolling, probably for many species, although the cost for searching females may be increased because of the higher proportion of time on the wing. Little site fidelity must be apparent. However, patrolling males could cover larger areas of the habitat and are more likely to encounter females. Then, males have to fly continuously through a large area, along tracks or forest margins, looking out for females. Watanabe (1979a) described the flyway of males in the swallowtail butterfly, *Papilio xuthus*, along the forest margins, which is the habitat for the host plants of the larvae.

Courtship Behaviour

Although sexual communication in butterflies involves the use of multiple signals at different phases of mate recognition and choice, constituting a complex scenario (Klein and de Araujo 2010), initial attraction usually takes place by visual channels (Scott 1972). The view is that butterfly wing pigment patterns are important for mate recognition and female choice. Females of the pipevine swallowtail butterfly, *Battus philenor*, preferentially mate with males that display a bright and highly chromatic blue on their dorsal hindwing (Rajyaguru et al. 2013). The information contained in the male visual signals must alter or control the female behaviour, and

probably vice versa. If the development of the ornament is correlated with male genetic quality, females must gain the benefits in good genes for offspring, such as superior disease avoidance, as well as in receiving high nutrient-rich materials for themselves. However, Kemp et al. (2008) found that in the alfalfa butterfly, *Colias eurytheme*, ultraviolet (UV) coloration of males is not correlated with the quality of the spermatophore produced. In addition, pheromones are used for courtship behaviour to facilitate mating by males of some species, releasing from specialised scent organs such as hair-pencils, coremata and modified scales. Scent substances on the wings have been found in both sexes of the common grass yellow, *Eurema hecabe*, and both pheromones could be used in male discrimination (Takanashi et al. 2001), although only male sex pheromones elicit female mating behaviour in many butterfly species (Rutowski 1978).

Because of the differences in the relative positions of the female and male wings during the courtship flights in the pipevine swallowtail butterfly, *Battus philenor*, the perceived colour of the male's dorsal wing surface changes dramatically over a range of wavelength (Rutowski et al. 2010). For a given position of receiver and light source above a wing surface, both the brightness and the chroma of the reflection seen by the viewer must change as the wing moves during a wingbeat cycle. Silberglied and Taylor (1978) clarified that males of the alfalfa butterfly, *Colias eurytheme*, and of the clouded sulphur, *Colias philodice*, search for females visually, using UV reflectance, and that some pheromone is vital to the females for successful courtship.

In the pale clouded yellow, *Colias erate*, which showed the characteristics of patrolling species, Watanabe et al. (1997) showed that males can easily encounter females when they fly around the habitats, probably using visual cues. Irie and Watanabe (2009) also reported that newly emerged females are, in general, easily found by mate-searching males on the wing in the early morning. Such virgin females simply accept courting males and then copulate (Fig. 2.3). When the mated female flying accepts the courtship of the male, she alights on the vegetation, remaining motionless on the perch site. The male follows her, alights next to the female and bends his abdomen to the tip of the female's abdomen for copulation. When the female showed mate refusal behaviour because of recent copulation, males stopped their courtship behaviour and flew away from her (Hasegawa and Watanabe 2008). Consequently, in the daytime almost every flying female seems to be unavailable for searching males. Mated females often showed a pierid-specific mate refusal posture at the perching site (wings spread, abdomen nearly vertically upturned) or a so-called ascending flight followed by the courting males. However, dissection of females captured in the field shows that females mate a couple of times during their lifespan (Nakanishi et al. 1996), indicating that mated females do not always refuse males. Therefore, males have a chance to mate with experienced females as well as with virgin females.

There are species-specific ratios of encounter rates between the sexes. For example, virgin females of the small heath butterfly, *Coenonympha pamphilus*, search out male territories and show a lengthy solicitation flight, whereas mated females avoid being detected in male territories (Wickman 1986). The ringlet

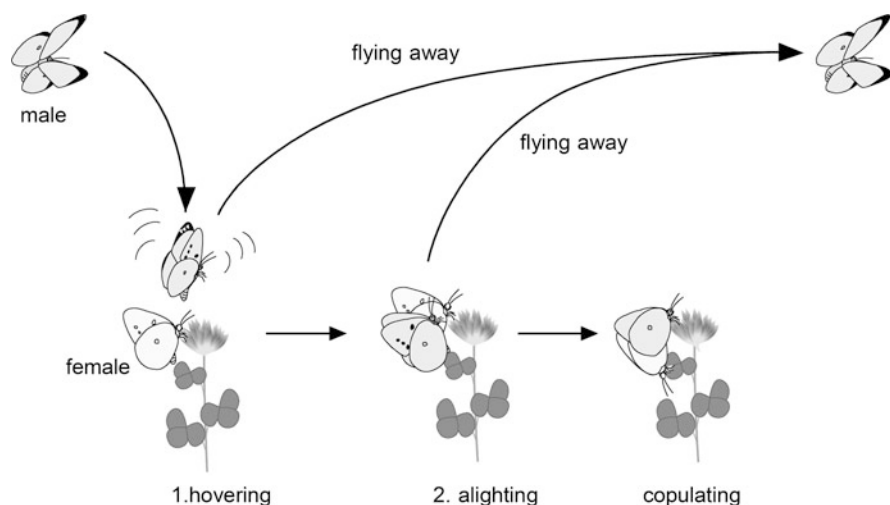


Fig. 2.3 Schematic representation of the courtship behaviour of the male pale clouded yellow, *Colias erate*, to the presented female (Hasegawa and Watanabe 2008). 1 A flying male visits the presented female and starts to hover in front of the female. 2 The male alights on the edge of the female's wings, walks down, and bends the abdomen towards the female; the male and the female then copulate. In each stage, when the males abandon copulation, they fly away from the female

butterfly, *Aphantopus hyperanthus*, also shows a similar shift in female behaviour after mating (Wiklund 1982). In the daytime, on the other hand, males of the pale clouded yellow, *Colias erate*, actively patrol on the wing, searching for females in open grasslands (Watanabe and Imoto 2003). They show powerful flying with rapid wingbeats, and cruise at a low height above the vegetation all day (except around noon, when the temperature is too high for flying) to search for females, with only a few intermittent periods of resting and feeding, while females perch in the vegetation and fly much less frequently. When males find a female perching or flying, they visit the female to start courtship behaviour. Males hover around the perching female, or near the flying female, inhibiting her flight course, probably using some pheromones.

In the Lepidoptera, there are many reports on the sequence of nuptial communication between males and females before copulation, including the female's response to the male's courtship behaviour (Wiklund 2003). As shown in Fig. 2.4, the female posture of exposing her abdomen from between the hindwings towards the male during the courtship behaviour has often been reported as a signal of female receptivity in butterflies such as the buckeye butterfly, *Precis coenia* (Scott 1975), the wood white, *Leptidea sinapis* (Wiklund 1977), the little sulphur *Eurema lisa* (Rutowski 1978), the alfalfa butterfly, *Colias eurytheme* and *Colias philodice* (Grula et al. 1980) and the pale clouded yellow *Colias erate* (Watanabe and Irie 2011). On the other hand, in the pipevine swallowtail, *Battus philenor*, males that have greener hindwings might enjoy a greater mating success because the dorsal iridescent area of the hindwing in males is a sexual signal (Rutowski

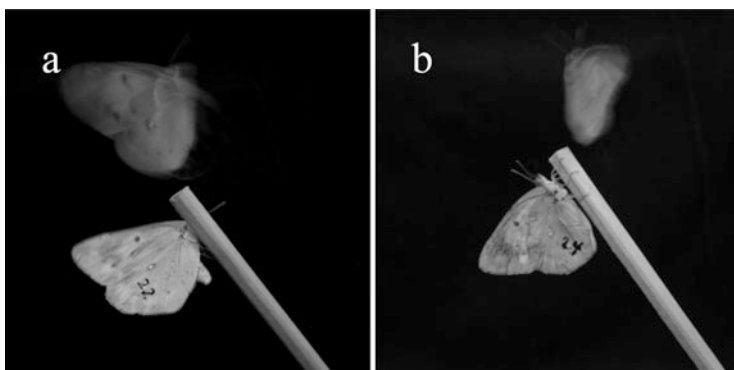


Fig. 2.4 Female response to the fluttering male in the pale clouded yellow, *Colias erate*. (a) The virgin female showed her exposed abdomen towards the male. (b) The unreceptive female did not expose the abdomen. (From Watanabe and Irie 2011)

et al. 2010). During aerial courtship manoeuvres in the pipevine swallowtail (Rutowski et al. 1989), the male positions himself below the female and flies up in front of her repeatedly, clearly displaying his dorsal wing surfaces to her. Rutowski and Rajayaguru (2013) clarified that ablation of male dorsal iridescence significantly reduced male mating success with virgin females.

Courtship proceeds along the behavioural sequence in which male and female recognise each other as a suitable mate. Successful copulation occurs only after the typical courtship behaviour is achieved (Silberglied and Taylor 1978). More specifically, male and female behaviour mutually release the next behaviour of both sexes. To evaluate female receptivity during courtship behaviour, males exploit the positive female behaviour. The female abdominal extension towards the male is an indication of female receptivity, and it may be a releaser of male alighting. Birch et al. (1989) demonstrated that when the females move off, away from male courtship behaviour, the males fly away from the female without any attempt for copulation. Therefore, butterfly females readily repel male attempts so that antagonistic coevolution between males and females does not escalate.

Copulation

Mating begins with the male positioning himself side by side with the stationary females. If the female, particularly of the pierid butterflies, is not receptive, she performs a specific mate refusal posture. If the female keeps her wings folded, leaving the tip of the abdomen physically reachable for the male, mating ensues (Watanabe and Irie 2011). Then, the male inserts his penis (aedeagus) into the vulva (vestibulum) of the female. To insert successfully, he has to engage her abdomen with a special locking device. At the tip of his abdomen there is a fixed (Fig. 2.5), hook-like structure (the uncus) with two lateral arms (valves) that close together to

Fig. 2.5 A pair of silver-studded blue, *Plebejus argus*, copulating on the grass



give a strong grip, as described by Vane-Wright (2003). Once firmly locked together, the tip of the aedeagus is then exerted between the valves to reach into the vestibulum, for entering the vagina proper. The male thus transfers his sperm to the female.

The mating ability of males on the day of eclosion is generally low, and the 1-day-old males start to search for females to copulate. Although the species-specific courtship behaviour performed by males is needed for females to accept the male in copulation, copulation by pairs of some swallowtail butterflies, mainly *Papilio* spp., *Byasa* spp. and *Battus* spp., can be artificially done by human hands, a so-called hand-pairing technique (West 1983; Scriber and Lederhouse 1988; Watanabe and Hirota 1999). The tips of the male and female abdomens are brought together, squeezed so the male opens his claspers and then the male is joined with the female (Fig. 2.6).

Even in the monandrous species, few virgin females are generally collected from a high density of the local population in the field, suggesting that females mate when encountering males soon after eclosion. In the black-veined white, *Aporia crataegi*, Watanabe (1978) observed that males are able to locate female pupae that are just emerging on the host tree of the larvae (Fig. 2.7). They scramble to approach the newly emerged females even though their wings are not yet fully enlarged and hardened. Some of the males even mount the pupa. The aim of males seems to be the first to copulate with the female, so that these females have no control over who will mate with them, although they can re-mate. Similar phenomena of such a 'pupal rape' were described in *Heliconius* spp. and the New Guinea yellow birdwing by Vane-Wright (2003).

Fig. 2.6 Schematic representation of the hand-pairing technique for the swallowtail butterfly, *Papilio xuthus*



Fig. 2.7 A male (circle) flying around a twig of the host tree of the black-veined white, *Aporia crataegi*. Pupae are on the defoliated twigs



There is actually very little variation in duration in copulation when the pair is not disturbed by conspecifics, or predators including birds, other insects and humans. Rutowski and Gilchrist (1986) suggested that duration of copulation might be relatively long as a result of the mechanical problems of filling the bursa copulatrix. Successful copulations in the swallowtail butterfly, *Papilio xuthus*, lasted about 1 h (Fig. 2.8), by which time the spermatophore had been structured in the bursa copulatrix and substantially filled with white creamy secretion and sperm (Watanabe et al. 2000). The copula duration in many species is at least an hour for



Fig. 2.8 Copulation of the swallowtail butterfly, *Papilio xuthus*

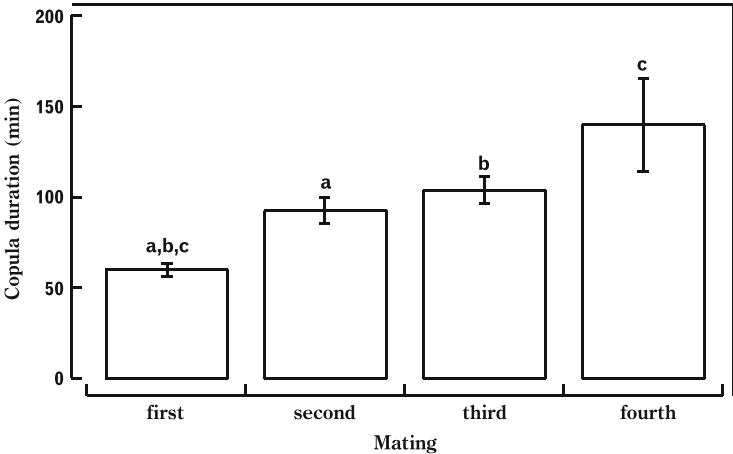


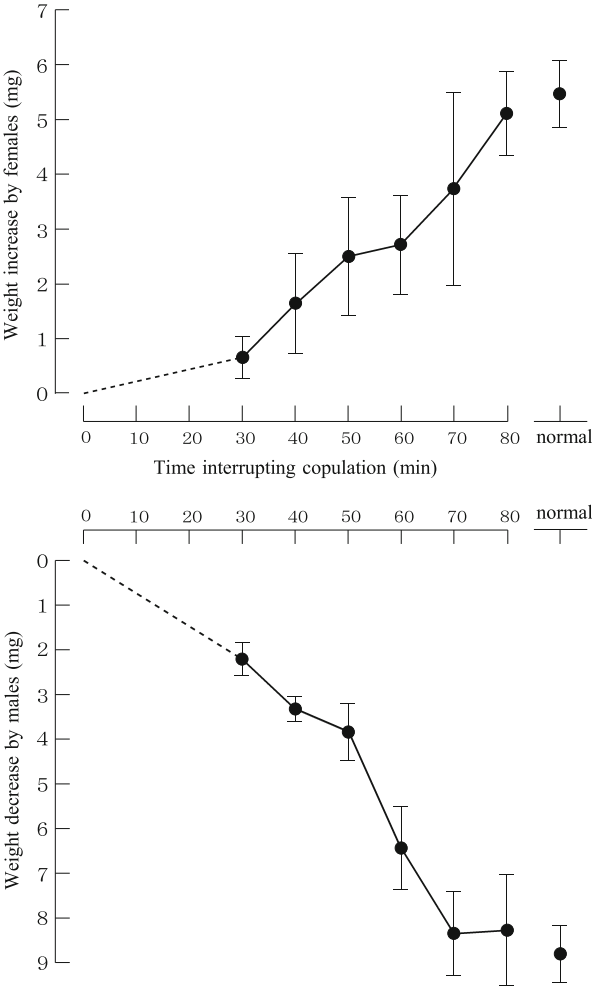
Fig. 2.9 Mean copula duration with males of the cabbage white, *Pieris rapae*, from the first to the fourth mating. Bars are standard errors. The same letters are significantly different at the 1 % level by Mann–Whitney *U* test. The number of matings exerts a positive effect upon copula duration by Kendall test (Watanabe et al. 1998b)

swallowtail butterflies (Watanabe and Hirota 1999) and for the cabbage white, *Pieris rapae* (Watanabe et al. 1998a), and sometimes much longer, as in the pale clouded yellow, *Colias erate*, because of harassment by the single males (Watanabe et al. 1997). Watanabe et al. (1998b) clarified that male cabbage whites increased copula duration with the number of matings (Fig. 2.9). Thus, the males that mated three times (they must be old at that time) copulate for more than 2 h. Caballero-Mendieta and Cordero (2013) also reported that the copulation duration for relatively large males of the monandrous pierid butterfly, *Leptophobia aripa*, was 83 min, 93 min and 491 min, for his first, second and third copulation, respectively. Kaitala and Wiklund (1995) showed that copulations with recently mated males of the

green-veined white, *Pieris napi*, can last up to 24 h, suggesting that the increase in the number of matings may carry substantial time costs. On the other hand, the copula duration of virgin males of the monandrous small copper, *Lycaena phlaeas*, is about 15 min (Watanabe and Nishimura 2001), and for the monandrous speckled wood butterfly, *Pararge aegeria*, it is about 20 min (Vande Velde et al. 2011).

Substances are passed to the female continuously throughout copulation. This deposition represents approximately 7 % of the male’s body mass for the cabbage white, *Pieris rapae* (Watanabe and Sato 1993) and the clouded sulphur, *Colias philodice*, or the alfalfa butterfly, *Colias eurytheme* (Marshall 1985). Spermato-phore transmission from male to female during copulation must result in weight loss from the male body and the corresponding weight increase of the female body. Figure 2.10 shows that during copulation the weight increase in the female roughly

Fig. 2.10 Weight increase by mated females and loss by the mated males during interrupted and uninterrupted (= normal) copulations (\pm SE) in the cabbage white, *Pieris rapae* (Watanabe and Sato 1993)



coincides with the weight loss in the male. In an experiment with the cabbage white, there was no detectable weight loss by mated males 20 min after the onset of copulation, but thereafter male weight began to decrease. The loss was about 8–9 mg in 70 min or 80 min of interrupted copulation, each of which was not significantly different from that of normal copulations. The weight of the mated females also had not increased at 20 min after the onset of copulation, suggesting that little transfer from male to female occurred during the first 20 min of the copulation. The weight increase at 80 min of interrupted copulation was not significantly different from that of normal copulation. The mean wet mass of the spermatophore was about 5.8 mg, and the mean body mass of the virgin males and virgin females was 79.0 mg and 81.6 mg, respectively. Consequently, the mass of a spermatophore, plus the appendix bursa contents, represents 7.3 % and 7.1 % of the body mass of males and females, respectively. On the other hand, 0.83 % and 0.94 % of male body weight is invested in the ejaculate produced by the virgin male of the monandrous mountain white, *Leptophobia aripa* (Caballero-Mendieta and Cordero 2013) and the speckled wood, *Pararge aegeria* (Bissoondath and Wiklund 1995), respectively.

Although the duration of copulation in butterflies seems to be under male control (Wickman 1985), one of the possible costs of mating incurred by males in transferring spermatophores is time. Many butterflies try to decrease copula duration against the risk of predation (Rutowski 1984), although prolonged mating has a function other than spermatophore transfer alone irrespective of size. In pierid butterflies, differential transfer rates result from behavioural competition among males (Svård and Wiklund 1988).

Thibout (1979) mentioned that copulation is not limited to a mere transfer of sperm from males to females, but is actually a complex process in which various behavioural sequences should be distinguished and followed by various physical effects on the female. Then, the important aspects of the copulation are as follows:

- (a) Precopulatory sexual behaviour
- (b) Introduction of the male genitalia into the female
- (c) Transfer of sperm and the various male secretions into the bursa copulatrix
- (d) Presence of the spermatophore and swelling of the bursa copulatrix
- (e) Presence of sperm and the male secretions in the bursa copulatrix
- (f) Activity of sperm in the spermatheca after sperm migration from bursa copulatrix to spermatheca

Spermatophore

After the onset of copulation, particularly for the swallowtail butterflies, *Papilio* spp., and pierid species, the bursa copulatrix, initially a thin, flat ellipse, grows rapidly in volume and weight, and becomes oval at about the time the spermatophore is structured (Fig. 2.11). For the cabbage white, *Pieris rapae*, the weight of the bursa copulatrix in virgin females is 0.84 mg. When the weight of the ejaculate

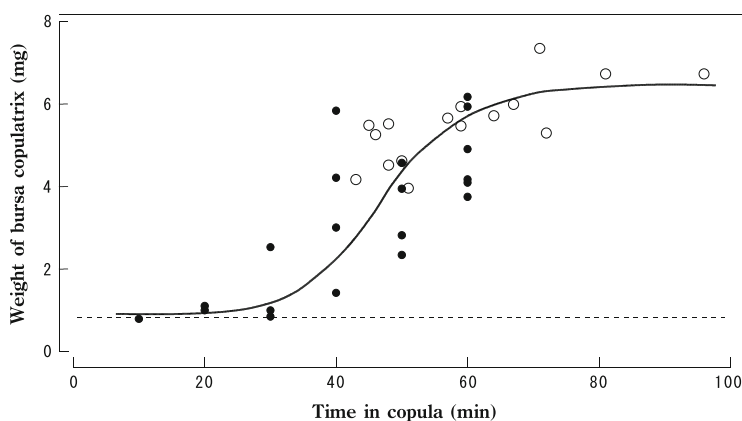


Fig. 2.11 Change in weight of bursa copulatrix of females after interrupted (*closed circles*) and uninterrupted (*open circles*) copulations in the cabbage white, *Pieris rapae*. Dotted line shows mean weight of the bursa copulatrix of virgin females. (From Watanabe et al. 1998a)

can be assessed by subtracting the weight of the bursa copulatrix of virgin females from the whole weight of the bursa copulatrix of mated females, there is no detectable change in the weight of ejaculate at 20 min after the beginning of copulation (Watanabe et al. 1998a). The weight of the ejaculate begins to increase thereafter. Then, during copulation, the male secretions from the ductus ejaculatorius and accessory glands are transferred serially to the bursa copulatrix. The ejaculate is white gel mainly observed on the tip of the penis 10 min after the beginning of copulation. There is no spermatophore capsule in the bursa copulatrix after 20 min in copulation. Then, at 45 min of copulation, the ejaculate weight is not significantly different from that observed in cases of uninterrupted copulation.

For the pipevine swallowtail, *Battus philenor*, the simplex of virgin males is a long tube and contains white, granular material and clear, pasty material (Sasaki et al. 2015). After the first mating, then, the simplex is shorter and lower in mass, and neither the white, granular substance near the aedeagus nor the clear pasty substance in other parts of the simplex are found in the simplex. Their relative position in the simplex requires that the white material must be placed first in the bursa copulatrix, followed by the clear substance. In the swallowtail butterfly, *Papilio xuthus*, spermatophore and accessory substances are transferred to the bursa copulatrix separately, and the accessory substance is located behind the spermatophore (Watanabe et al. 2000).

The spermatophore proper represents a balloon-like or a teardrop-shaped structure with a swollen corpus and a tube-like column with an opening at its tip (Fig. 2.12). In addition, gel-like accessory substances are transferred to the bursa copulatrix. Ejaculates including both spermatophore and accessory substances are physiologically costly for males to produce, with ejaculate mass ranging from 1.4 % in the speckled wood, *Pararge aegeria* (Svård and Wiklund 1989) to 15.5 % in the Labrador sulphur, *Colias nastes* (Rutowski et al. 1983). Males of the green-veined

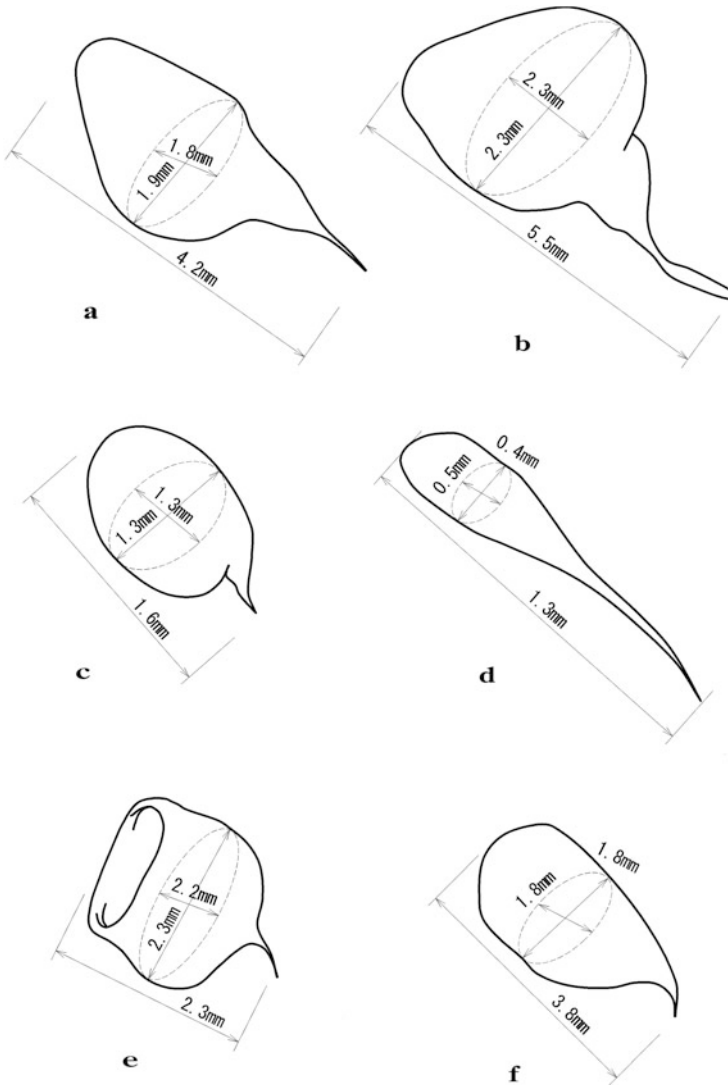


Fig. 2.12 An intact spermatophore just after termination of copulation in the common straight swift, *Parnara guttata* (a); the common yellow swallowtail, *Papilio machaon* (b); the cabbage white, *Pieris rapae* (c); the small copper, *Lycaena phlaeas* (d); the painted lady, *Vanessa cardui* (e); and the Japanese nymphalid butterfly, *Lethe diana* (f)

white, *Pieris napi*, have been observed to transfer a spermatophore corresponding to 23 % of their body mass, and its size is correlated with male body size (Fosberg and Wiklund 1989). The fresh weight of the spermatophore just after termination of copulation is about 6 mg in the swallowtail butterfly, *Papilio xuthus* (Watanabe et al. 2000) and the cabbage white, *Pieris rapae* (Watanabe et al. 1998b).

Although in most invertebrates females are usually larger than males, in some butterfly species sexual size dimorphism increases with the degree of polyandry, with males being larger than the females (Wiklund and Forsberg 1991). Across the species, such a relative male size increases with female polyandry, in relationship to the positive association between spermatophore size and male size (Bissoondath and Wiklund 1997). Because the large spermatophore is beneficial by both delaying female re-mating and increasing female reproductive output, large-size males must have a high advantage over small-size males.

Because sperm is stored in the duplexes that unite to form the simplex, sperm must be transferred to the female after transfer of the clear pasty substance. Thus, sperm are the last materials transferred to the female reproductive tract in the cabbage white, *Pieris rapae* (Watanabe and Sato 1993) and in the alfalfa butterfly, *Colias eurytheme* (Rutowski and Gilchrist 1986). At this time, the spermatophore contains immobile eupyrene sperm bundles and an actively free apyrene spermatozoa in the swallowtail butterfly, *Papilio xuthus* (Watanabe and Sasaki 2010) and in the cabbage white (Watanabe et al. 1998a, b).

Sperm stay in the spermatophore for several hours after copulation, during which time eupyrene sperm bundles begin to disappear with an increase in free eupyrene spermatozoa in the swallowtail butterfly, *Papilio xuthus* (Watanabe et al. 2000). Sperm of the large white, *Pieris brassicae*, starts to migrate to the spermatheca 5.5 to 8 h after copulation (Tschudi-Rein and Benz 1990). The spermatophore must be a site of sperm maturation during the period (Osanai et al. 1987). Accessory gland products in the spermatophore from the male have been shown to function in sperm activation (Leopold 1976). Sugar content in the spermatophore might contribute to sperm survival during this period (Watanabe and Sato 1993).

In some species, a spermatophore contains nutrients for the female that may support somatic maintenance (Boggs and Gilbert 1979) or increase her fecundity (Watanabe 1988). Males that can produce larger spermatophores are expected to have greater fertilisation success, especially in the polyandrous species (Parker 1982), because large spermatophores increase the length of the refractory period of the female, that is, the time during which the female is unreceptive to male courtship following copulation (Sugawara 1979). In addition, males might gain sperm priority by transferring a large spermatophore in some species (LaMunyon and Eisner 1994). However, the advantage of high sperm number might be relatively small in butterflies because of evidence that in many species, including the swallowtail butterfly, *Papilio xuthus* (Watanabe and Sasaki 2010), sperm mixing in the sperm storage organ rarely occurs in females mated more than once, resulting in the bimodal distribution of paternity or in the precedence of the last male sperm.

Estimation of Age

Realised adult longevity is a crucial life history trait because it will influence how many times an individual can engage in reproductive activities. Longevity must be essential to increase fitness for both sexes. Thus, males as well as females can have

the chance to mate repeatedly during a long lifespan, as the mortality rate increases by external factors such as predation, starvation or drought with age. Age-specific fecundity pattern in relationship to female longevity has been reported in the laboratory by Labine (1968). Because adults of species that have one generation in a year show ageing relatively similar to each other because of the similar emergence period, each adult collected in the field must not be so considered in age for study. However, in the case of species that have more than two generations in a year, adults from different generations could coexist from summer to early autumn, because of adult longevity longer than the length of the larval period, weather conditions and so on. Consequently, there are many adults of various ages flying during the active flying seasons.

Wiklund et al. (2003) stated that all other things being equal, selection for extended male longevity should be stronger in polyandrous mating systems in which males are allowed to mate with females throughout the female flight period than in monandrous mating systems in which male mating opportunity is considerably more restricted in time. Although females of some polyandrous species must become unreceptive for a number of days after mating, females receptive for copulation must appear throughout the flying season. In other words, because females mate multiply throughout their lives, males could increase the number of matings with such receptive females throughout their own lives. On the other hand, for monandrous species, male mating success may not be expected to be associated with lifespan because the time period of the appearance of receptive females is rather short. Therefore, the male mortality rate seems to be greater than the female mortality rate in monandrous species.

Applying a mark-and-release survey to field populations has clarified many population parameters including estimated longevity, by using the Jolly–Seber method and the Manly and Parr method for various butterfly species such as the checkerspot butterfly, *Euphydryas editha* (Ehrlich 1965) and the black-veined white, *Aporia crataegi* (Watanabe 1978), both of which have one generation in a year. Population parameters of the black swallowtail butterfly, *Papilio protenor* (Fig. 2.13) and of *Papilio helenus* in summer generations were surveyed with the consideration

Fig. 2.13 A male of the spangle, *Papilio protenor* (black swallowtail butterfly), feeding on the flowers of the bushkiller, *Cayratia japonica*. (Photograph by T. Kasui)



of age of each adult captured by Kiritani et al. (1984). Nozato et al. (1985) proposed a survivorship curve of adults of black swallowtail butterflies wherein low and high mortality is found during young and old ages, respectively. With the exception of species that pass the winter as adults, most butterflies commonly live 2 or 3 weeks, unless overcome by accident or killed by predators. Ehrlich and Gilbert (1973) reported an exceptional species; the longevity of the ethilia longwing, *Heliconius ethilla*, was 6 months in the tropics.

After eclosion, every butterfly begins to show age. The colours of body and wings start to fade. Watanabe and Ando (1993) suggested that UV reflectance for the cabbage white, *Pieris rapae*, decreases with age after emergence. In general, the wings become rubbed and frayed from encounters with other insects including butterflies, the complex structure of vegetation, spider webs, and all manner of little accidents. There is no opportunity for repair, because there is little or no cell division, and no growth in the adult butterfly, other than those processes involved with reproduction. Therefore, the butterfly will perish at least after some weeks. As the males of most species age, probably with the experience of matings, in general, they might produce smaller and smaller spermatophores. Then, one of several reasons for receptive females to reject a male is that he is old. Consequently, the reward for mating with an old male may simply be too low. Females need to be sure of the male's relative age, as well as recently mated males, mainly by noting the wing wear of males.

To clarify the internal condition of butterflies as well as population structure, the age of each butterfly collected in the field has to be estimated. Because colouration degrades with age and wing wear, on the basis of the degree of wing wear, several age groups can be classified for the swallowtail butterflies, *Papilio polytes* (Watanabe 1979b), *Papilio xuthus* and *Papilio machaon* (Watanabe and Nozato 1986), and the pierid butterflies, *Pieris canidia* (Ando and Watanabe 1992), *Pieris melete* (Ando and Watanabe 1993) and *Pieris rapae* (Watanabe and Ando 1993). The five age groups (FF, F, B, BB and BBB) can generally be classified. An adult of FF group is defined as a recently emerged one with intact wings and lustrous scales. In addition, the bright coloration in FF group might indicate a low probability of previous mating. Adults of F group have still fresh wings with fine tears but less lustrous scales. Adults of B group have wing tears with frayed scales. Adults of BB group show notched tears and highly frayed scales. Wings of adults of the BBB group are broken or have extensive tears and highly frayed scales (Fig. 2.14).

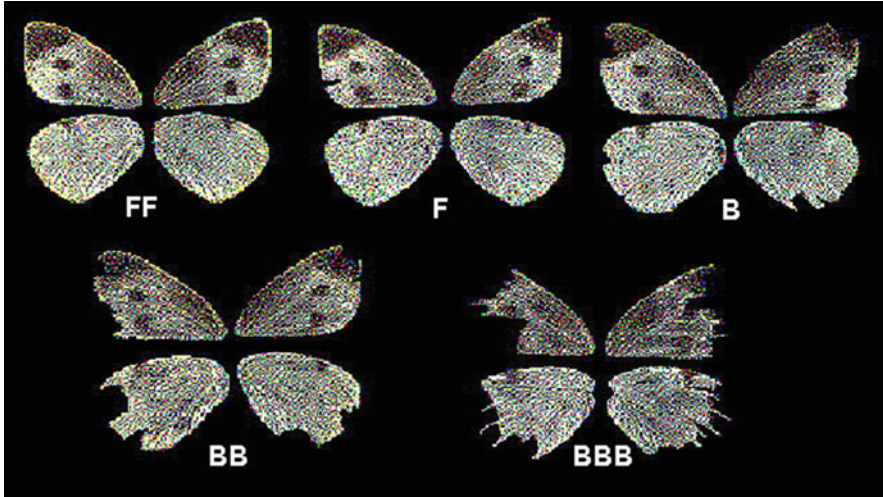


Fig. 2.14 Five age classes for the cabbage white, *Pieris rapae*

Lifetime Mating Frequency

Because a key determinant of the intensity of sexual selection is the variance in the reproductive success of individuals in natural populations, it is important to determine the lifetime mating number of both sexes. It had been believed that females of some species with a very short longevity usually mate only once, whereas females with a long period for oviposition will mate several times. The reason might be to replenish their store of sperm, to obtain more viable sperm, or to gain additional nutrients that will help to produce more eggs or allow somatic maintenance. Indeed, Vane-Wright (2003) stated that females of some milkweed butterflies mate frequently to obtain continual supplies of alkaloids, both for the protection of their eggs and for their own survival, because the alkaloids are highly unpalatable to many predators, including birds. Therefore, it is important to examine multiple matings in females that have mated multiple times to different males throughout their lifespan, that is, true polyandrous matings. Repeated matings, defined as mating the same male several times, are not common in butterfly species.

Mating success of males can only be documented by seeing them in copula. Consequently, male mating success in natural populations can often be described only by observing copulations, such as in lekking animals (Fiske et al. 1998) and damselflies (Fincke 1982). Males of most butterfly species probably mate as many times as they can. Some males copulate several times before they die, but unsuccessful males may die as virgins. Therefore, males would show a higher variance in reproductive success than the females. In addition, the expected number of matings by males correlates with the degree of polyandry exhibited by the species (Karlsson 1996); this contrasts with females, most of which mate at least once.

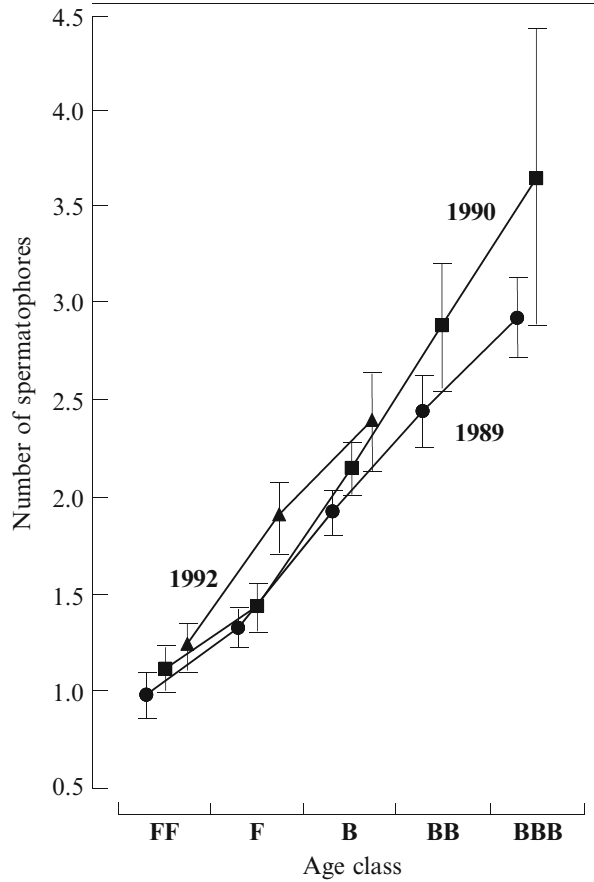
The lifetime mating success of wild females is often easier to assess than that of males. Females of some insects are wounded by male external spines during copulation, and these wounds can indicate mating history (Crudgington and Siva-Jothy 2000). In butterflies, however, females receive a spermatophore during a single copulation without any apparent injury by the male, and the remains of individual spermatophores can be found in the bursa copulatrix and counted even after they are drained of their contents (Burns 1968). The bursa copulatrix of females is balloon shaped, and it can expand to several times its original size depending on the number of spermatophores. Thus, female butterflies have considerable capacity for the storage of multiple spermatophores (Parker 1970).

Spermatophore counts of wild-caught female butterflies, which are routinely used to assess female mating history, indicate that the females of many species mate more than once (Lederhouse 1981). The reason for female polyandry has been believed to be that the sperm from their previous partners have been fully utilised. However, a number of studies have shown that they often re-mate before sperm exhaustion and that multiple mating occurs in the wild in many butterfly species (Stern and Smith 1960). Multiple matings, accordingly, seem to be the rule in most butterfly species, although females of the wood white, *Leptidea sinapis* (Wiklund 1977), the small copper, *Lycaena phlaeas* (Watanabe and Nishimura 2001) and the pipevine swallowtail, *Battus philenor* (Konagaya et al. 2015) are monogamous. However, the number of spermatophores in many studies was “the average mating number” for all females collected, ignoring female age.

The lifetime mating number of females should be estimated by the number of spermatophores in the bursa copulatrix, considering from that for the youngest to the oldest females. The mating number of females increases with the age group. Thus, the relationship between estimated female age and the number of spermatophores in the bursa copulatrix could estimate the lifetime mating number. The lifetime mating number of the black swallowtail butterflies (*Papilio helenus* and *Papilio protenor*) in a summer generation is 3 to 4 (Watanabe et al. 1986). For the swallowtail butterfly, *Papilio xuthus*, the lifetime mating number was about 3 for spring and summer generations (Watanabe and Nozato 1986). For the cabbage white, *Pieris rapae*, in the summer generation the lifetime mating number was about 3.5 for every year. The average number of matings in females of the polyandrous comma butterfly, *Polygonia c-album*, which is univoltine and overwinters in the adult stage, is 2.5 (Svärd and Wiklund 1989). Konagaya and Watanabe (2015) showed that the autumn-morph females of the common grass yellow, *Eurema mandarina*, mated once for their own somatic maintenance before overwintering and then re-mate for fertilisation in oviposition after overwintering. On the other hand, Tatar (1991) reported that the females of the pipevine swallowtail, *Battus philenor*, in early-spring and late-spring generations in California, contained 1.1 and 1.3 spermatophores, respectively. In summer generations of the pipevine swallowtail in Arizona, Konagaya et al. (2015) found that the first mating of females occurred very soon after eclosion but that the lifetime mating number was 1.4 without any change in the volume of spermatophores.

As shown in Fig. 2.15, the number of matings in the cabbage white, *Pieris rapae*, increased with age in each year, and the pattern of change is similar in all 3 years

Fig. 2.15 Change in the mean number of spermatophores in wild-caught females of the cabbage white, *Pieris rapae*, for five age classes. Circles, squares and triangles indicate data for 1989, 1990 and 1992, respectively. Each bar represents SE. (From Watanabe and Ando 1993)



(Watanabe and Ando 1993). As the estimated male density changed from year to year in the habitat, as well as the fluctuation of weather conditions, the daily number of active males searching for mates must differ during the 3 years. Therefore, a species-specific lifetime mating number of females might be proposed, indicating that the lifetime mating number might be controlled by the female herself, not by the population density, male activities or other environmental factors.

Discussing the lifetime mating number of females seems to refer to a females-win scenario, wherein further increase in male defensive ability is checked either by a lack of additive genetic variation or by direct/indirect natural selection acting upon male ejaculate expenditure (Välimäki and Kaitala 2010). A mating number close to the female optimum must follow, if both the optimal female mating number and its potential to evolve are sufficiently high, and if the selection for increasing spermatophore depletion ability is intense enough. A three-way conflict among female reproductive physiology, male defence and male offence has been considered for the explanation of control of the female mating number.

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