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Multiple paternity in the meadow spittlebug *Philaenus spumarius* (L.) (Homoptera: Cercopidae)

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Abstract. Incidence of multiple paternity was investigated in the British meadow spittlebug *Philaenus spumarius* by using distinctive polymorphic colour patterns, which are under genetic control of 41 crosses set up in the laboratory; 25 were successful in producing together 737 progeny. Direct evidence of multipaternal progeny was detected in 13 crosses. Twelve crosses involved at least two males' paternity, and one cross clearly demonstrated triple paternity in the resultant progeny. According to results, the last male may father up to 91% of the progeny, indicating the last male sperm precedence (Mann-Whitney *U*-test, $P<0.01$) in *Philaenus spumarius*. Although the number of progeny (range 2-135, mean 29.48) and total copulation duration (range 6–63 hours, mean 29.00) of the multiply mated females showed variation, there was no correlation between these two variables ($r= -0.292$, $N=25$, $P>0.05$). Average number of the progeny did not show significant difference among the females that had two or more copulations (Kruskal-Wallis, $H=3.00$, $df=2$, $P>0.05$). However, the mean copulation duration of the females that had more copulations were highly significantly longer than those had less copulations (one-way ANOVA: $F_{(2,22)}=P<0.01$).

Key words: sperm competition, multiple paternity, last male advantage, *Philaenus spumarius*, polymorphism.

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I. INTRODUCTION

Sperm competition has become a phenomenon after PARKER's pioneering review in 1970. It is known as the struggle between the sperms from two or more males to fertilise the eggs of a single female during one reproductive cycle. Sperm competition has been documented in many animal taxa ranging from arthropods to mammals (PARKER 1970; THORNHILL & ALCOCK 1983; SMITH 1984) including humans (BAKER & BELLIS 1994). The most spectacular examples occur in insects (RIDLEY 1988) and birds (BIRKHEAD & MOLLER 1992). A recent work of BIRKHEAD & MOLLER (1998) covers all major taxonomical groups.

Though females in many animals may mate multiply with the same male or different males, the mating frequency exhibits remarkable variation from species to species. This behaviour in some insects is extreme. For example, honeybee queens may mate up to fifty-three males during their nup-

tial flights (MORITZ et al. 1995). Double mating is almost a rule in field populations of some *Drosophila* species (LEVINE et al. 1980). In cases of multiple mating usually either the first male (ARRNQVIST 1982; WATSON 1993) or the last male (ARNQVIST 1988; PRICEet al. 1999) take advantage by fathering the resultant progeny. However, in most polyandrous species studied, it is the last male to mate that fertilises the majority of subsequent eggs. The proportion of the offspring fathered by the last male in polyandrous species shows variation and last male predominance sometimes exceeds over 90% as in several Zygoptera (WAAGE 1986), Heteroptera (SILLEN-TULLBERG 1981) and *Drosophila* (PRICE et al. 1999).

A number of mechanisms have been suggested for the reason of the first or last male sperm precedence. The most discussed and controversial mechanism is the sperm displacement resulting in removal of sperms of the first males from the female's genital tract by certain alternative ways. Evidence of sperm displacement has been demonstrated in the Odonata (WAAGE 1986), and it has been also supported by the recent findings such as in the beetle *Tenebrio molitor* (GAGE 1992), in the Dipterans *Dryzomia anilis* (OTRONEN & SIVA-JOTHY 1991) and *Scatophaga stercoraria* (SIMMONS et al. 1999). Other mechanisms involve the mating plugs of males that prevent entering the sperms to females' genital tract after the mating (DICKINSON & RUTOWSKI 1989; BAER & SCHMID-HEMPEL 1999). Copulation duration, which sometimes may last several days in some insects (CARROLL 1991), has also received attention related to sperm precedence and progeny size (SILLEN-TULLBERG 1981; JONG et al. 1993; LEWIS & AUSTAD 1994; PHORNHILL et al. 1999).

Several techniques have been developed for the detection of the paternity in the polyandrous species. Most of them merely rely on expensive and laborious molecular works including DNA marking (FAABORG et al. 1995; MORITZ et al. 1995; FJERDINGSTAD & BOOSMA 1998), labeled males with different radioisotopes (SIMMONS et al.1999) or sterilised males (SILLEN-TULLBERG 1981; ARNQVIST 1988; YAMAGISHI et al. 1992; LORCH et al. 1993), and enzyme polymorphisms (STILLE et al. 1986; WATSON 1993). Some of these include even very sophisticated methods in which sperms are marked with certain fluorescent particles in order to follow them under the magnification (PRICE et al. 1999). A few studies have utilised heritable morphological characters to assign the paternity (STILLE et al. 1986; BOITEAU 1988; SCHWARTZ et al. 1989; JONG et al. 1993; BAUR 1994).

Potential genetic and evolutionary advantages of polyandry have been a source of debate (TAYLOR 1967; WADE 1982; YASUI 1998). The evolutionary significance of multiple mating has been most often discussed in terms of the benefits of increased fitness (RIDLEY 1988; SWARD & WIKLUND 1989; BISSOONDATH & WIKLUND, 1995) and increased genetic variability (LOMAN et. al. 1988; MADSEN et al. 1992; WATSON 1993; BAUR 1994). Despite the severe arguments, numerous experimental studies have claimed that the benefits of multiple mating and multiple paternity were often associated with genetic compatibility of polyandrous species.

The meadow spittlebug *Philaenus spumarius* is a common homopteran occurring in most terrestrial habitats throughout the Holarctic region. The adults exhibit heritable colour/pattern variation on the dorsal (HALKKA & HALKKA 1990; STEWART & LEES 1996; YURTSEVER 2000a) and ventral sides (YURTSEVER 2000b). STEWART & LEES (1996) described thirteen colour/pattern morphs in *P. spumarius*, though eleven of these are most common. Of the 11 morphs, three are categorised as non-melanics — POP (*populi*), TYP (*typicus*), and TRI (*trilineatus*) are light straw coloured with dark mottling, or stripes. The remaining are melanics — MAR (*marginellus*), LAT (*lateralis*), FLA (*flavicollis*), GIB (*gibbus*), LCE (*leucocephalus*), QUA (*quadrimaculatus*), ALB (*albamaculatus*), and LOP (*leucopthalmus*), predominantly black or dark brown with different combinations of pale markings on the dorsal surface (Fig.1). The nomenclature and three letter abbreviations of these forms follow HALKKA et al. (1973).

The genetic basis of eleven morphs in *P. spumarius* has been studied extensively (HALKKA et al. 1973; STEWART & LEES 1988; YURTSEVER 1997). Seven alleles (indicated by the letters as given below) at a single autosomal locus (pigmentation locus "p") control the polymorphism, each one responsible for a single morph or group of similar phenotypes are as follows: p^t (POP+TYP), p^T

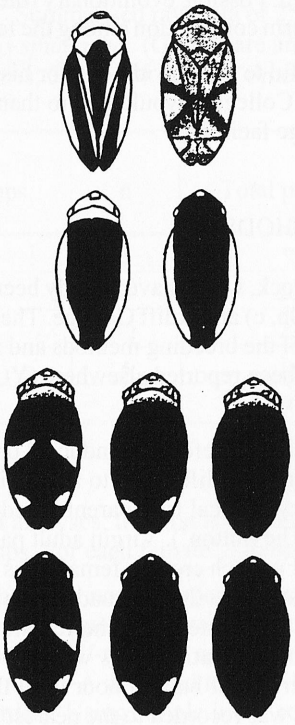

Fig. 1. Dorsal phenotypes of *Philaenus spumarius* commonly occurring in most populations. From left to right, the top row: TRI, and TYP. Second row: MAR, and LAT. Third row: FLA, GIB, and LCE. Bottom row: QUA, ALB, and LOP. Abbreviations are explained in text. (One of the most common morphs, the uniform pale yellow POP, which is a variety of TYP, is not given for convenience).

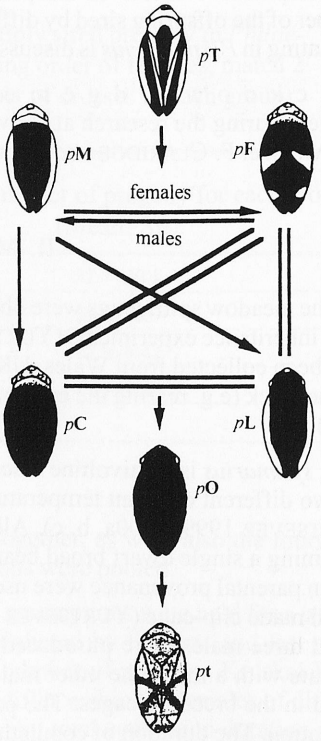

Fig. 2. Model of dominance hierarchy for eleven common phenotypes in some Welsh populations of *Philaenus spumarius* based on the crossing experiments (YURTSEVER 1997) and applied also in the present study. Arrows show direction of dominance, double lines show co-dominance. Each phenotype in the figure represents its own phenotypic group as described in text (updated version of HALKKA et al. 1990, and STEWART & LEES 1996).

(TRI), p^M (MAR), p^L (LAT), p^F (FLA-F), p^C (FLA-C+GIB+LCE), and p^O (QUA+ALB+LOP). As described above, FLA is expressed by either of the two alleles. Heterozygote combinations of $p^{L/C}$ and $p^{L/F}$ are also expressed as MAR in the phenotype due to co-dominant genetic relationships. Thus, the genotype of a particular FLA or MAR can only be traced by controlled inheritance experiments. Genetic background of these phenotypes is given in Table II.

The dominance hierarchies of eleven commonly occurring phenotypes concerning the seven-allele system have been established for Fennoscandian (HALKKA et al. 1973), several British (STEWART & LEES 1988; YURTSEVER 1997), Turkish (YURTSEVER 1999) and New Zealand populations (YURTSEVER 2000c). In some Welsh populations (YURTSEVER 1997), TRI is the top dominant phenotype, it is followed by melanics, and TYP is the bottom recessive (Fig. 2). In the two sexes there is a reversal of dominance between MAR and FLA: MAR is dominant to FLA in females but recessive to it in the males.

Although multiple mating has been studied across a wide variety of animal taxa, nothing is known about this behaviour in *P. spumarius*. In this paper, I present the incidence of multiple paternity in *P. spumarius* for the first time. The general aim of this study was to determine multiple pater-

nity patterns in polyandrous females of *P. spumarius*, based on inherited colour/pattern polymorphism. Number of offspring, total copulation duration of the females and predictable number of the offspring sired by different males were analysed. Possible evolutionary role of multiple mating in *P. spumarius* is discussed mainly in terms of sperm competition among the females.

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II. MATERIAL AND METHODS

The meadow spittlebugs were obtained from laboratory stock, which have already been used for other inheritance experiments (YURTSEVER 1997, 1999, 2000b, c) at Cardiff College. These insects have been collected from Wales, UK. Detailed descriptions of the breeding methods and maintaining the stock (e.g. rearing the eggs, nymphs and adults) have been reported elsewhere (YURTSEVER 1997).

P. spumarius is a univoltine insect and has a long life cycle. Therefore, I conducted breeding in the two different constant temperature rooms in order to reduce the life cycle to about six months (YURTSEVER 1999, 2000a, b, c). All insects were reared in cylindrical transparent breeding cages containing a single dwarf broad bean plant (*Vicia faba* cv. 'The Sutton'). Virgin adult pairs with a known parental provenance were used for genetical crosses. For each cross, a female was placed in a hand made clip-cage (YURTSEVER 1997), attached to the leaves of a dwarf broad bean plant. Two or/and three males were introduced to females at a time. Five minutes after the female began to copulate with a male, the other males were removed, and the copulating pairs were carefully released in the breeding cages. The pairs were checked at intervals of half an hour until the end of copulation. The duration of copulation in the individual pairs was recorded to the nearest full hour. After one or two-day intervals, another male was introduced to the same female. After the first mating, females usually resisted to subsequent matings, so that the length of intervals between the copulations sometimes took several days which resulted in great variation. Number of males mating with a given female also showed variation. These data caused difficulty in the analyses. If the copulation duration was not long enough (<hour), the same male was introduced to female again to ensure transfer amount of sperms sufficient for fertilisation and sperm competition. When the same male was not successful again, it was replaced with a new male. Where possible, individual males were introduced to mate at least twice, with the time between matings ranging from 1 to 15 days. In 25 crosses that produced progeny, 56 males were used and achieved 94 matings. Of these, 22 mated once, 28 mated twice, 5 mated three times, and 1 mated four times. In the experimental treatments, two or three different males were allowed to copulate with the same female to obtain clear evidence of multiple paternity. Males always displayed appetite behaviour for mating, in isolated cages they sometimes even attempted to mate with individuals of their own sex.

Homozygous TYP females were chosen for laboratory experiments, because this phenotype is recessive to all other phenotypes. However, some other dorsal phenotypes were used as female partners to prove that multiple paternity occurs throughout the morphs of *P. spumarius*. One dorsal-melanic and one TRI phenotype were used as male partners, because TRI is dominant over all other dorsal phenotypes in both sexes. Due to unavailability of the stock, some TYP males were also used (compare Table II). By using genetically distinctive polymorphic colour patterns as genetic markers, it was possible to detect multiple paternity in the resultant progeny. For convenience, POP+TYP, and GIB+LCE phenotypes are referred as TYP and LCE, respectively, throughout the paper.

The numerical data of 25 crosses are summarised in Table I. Crosses were grouped according to mating order of the females (e.g. mated once, twice or more). In order to obtain sufficient data for

Table I

Summarised raw data of 25 crosses, producing multiply sired progeny in *Philaenus spumarius*. (Groups are arranged according to mating order of females, mated 2 times, 3 times, 4 times, 5 times, and 6 times, n is number of crosses in each group)

Groups	n	Total progeny	Average number of progeny for each cross (Mean±SE)
2	3	98	32.7±20.1
3	4	205	51.2±28.9
4	12	227	18.9±6.42
5	5	160	32±10.6
6	1	47	–

statistical analyses, the first and second groups were pooled together, as were also the fourth and fifth groups. For each female, duration periods of all copulations were pooled.

Parametric or non-parametric tests were applied where the assumptions were held for a particular test on the data. To obtain proportions of the offspring fathered by the first and last males, Mann-Whitney *U*-test was applied on the predicted average number of progeny. Kruskal-Wallis one-way ANOVA was used to test whether the progeny sizes differ within the females of different mating orders. Using simple linear regression, effects of different total copulation duration on the progeny sizes of females were tested. A parametric one-way ANOVA was used in order to test whether copulation duration is associated with mating order. Multiple comparisons between the groups were made by using Tukey's HSD method.

Means were given ± one standard error and with sample sizes (n) where appropriate.

III. RESULTS

The study clearly revealed that multiple mating and multiple paternity occur in *P. spumarius* and multiply sired progeny can be segregated easily due to inherited polymorphism. Of 41 crosses set up for the study, 25 were successful in producing at least two offspring and together contained a total of 737 progeny (Table I). Family sizes were very variable (range 2-135, average 29.48). However, Mendelian segregation ratios were rarely possible to apply because of certain mechanisms in sperm competition (e.g. sperm replacement, sperm mixing; see Discussion). Moreover, there was an excess of TYP offspring in many crosses. The progeny from 12 crosses did not give clear evidence for the paternity, since different males may be claimed as father. Therefore, the details of these 12 crosses will not be discussed here.

The remaining 13 crosses clearly indicated that the progeny of polyandrous females were multiply fathered (Table II), because more than two alleles appear to be responsible for the occurrence of different phenotypes in the progeny. In P/15 and P/39, in which three different males mated with the same female, paternity of two different males is clearly detectable. It is unclear whether triple paternity could happen in these crosses. Nevertheless, P/34 provided good evidence for triple paternity, with four different phenotypes occurring in the progeny. All three males, heterozygous for TYP, contributed to the progeny. Four different phenotypes equally occurred in the progeny suggesting

Table II

Thirteen crosses giving clear evidence of multiple paternity in *Philaenus spumarius*. The last two columns are showing predicted number of offspring sired by the first and last males in 8 females that mated with two different males (TYP offspring ignored where appropriate, for details see the text)

Parents (genotypes are given in brackets)			Number of progeny (proportions are indicated in brackets)		
Cross no	Females	Males	Total	Fathered by first male	Fathered by second male
P/15	TYP*(tt)*	TYP LCE TRI	4 TRI 8 LCE 8 TYP		
P/16	TYP*(tt)*	TRI TYP	5 TRI 4 LCE 38 TYP	9(19)	38(81)
P/20	LAT*(Lt)*	LAT TRI	36 TRI 23 LAT 1 ALB 15 TYP	24(32)	51(68)
P/21	FLA*(FF)*	LCE TRI	20 TRI 9 FLA 7 LCE		
P/22	TYP*(tt)*	LCE TRI	37 TRI 19 LCE 79 TYP	19(34)	37(66)
P/26	TYP*(tt)*	TRI LCE	5 TRI 29 LCE 37 TYP	5(15)	29(85)
P/27	TYP*(tt)*	TRI LCE	3 TRI 30 LCE 39 TYP	3(9)	30(91)
P/28	TYP*(tt)*	LCE TRI	5 TRI 6 LAT 12 LCE 1 TYP	13(54)	11(46)
P/31	TYP*(tt)*	TRI LCE	3 TRI 20 LCE 23 TYP	3(13)	20(87)
P/32	LAT*(Lt)*	TRI LCE	4 TRI 1 MAR 16 LAT 8 LCE 8 TYP	4(31)	9(69)
P/34	TYP*(tt)*	TRI LCE	8 TRI 8 LAT 8 LCE 8 TYP		
P/39	TYP*(tt)*	LAT TRI LCE	3 LAT 4 LCE 1 TYP		
P/40	TYP*(tt)*	LAT TRI LCE	1 TRI 2 QUA 1 TYP		

an exact 1:1 ratio for each separate male. The remaining three crosses, in which females mated with three males, did not give direct evidence of triple paternity.

In 8 crosses, in which females mated with two different males, the proportion of the offspring sired by each male can be estimated. Although double paternity is evident in P/21, the proportion of offspring fathered by the first and last male cannot be estimated precisely, because genotypes of the FLA offspring are not predictable and may have been expressed by the two different alternative alleles (F and C alleles).

In P/16 and P/20, the proportion of offspring sired by the first and the second father can be evaluated exactly, because genotypes of female and male parents can be traced in the progeny. In P/16, the progeny consisted of 19% the offspring fathered by the first male and 81% fathered by the last male. These proportions for the first male and the last male in P/20 are 32% and 68%, respectively. Expected Mendelian ratios also seem to be applicable to these crosses. In P/16, 5 TRI and 4 LCE offspring suggest a 1:1 ratio, but a Chi-Squared Test is not applicable. However, it is clear that the last male TYP sires a large number of TYP offspring.

If the TYP progeny are ignored in 8 crosses in which females had double males to mate, it is possible to obtain proportion of the offspring sired by the first male (26%) and last male (74%) on the pooled data (Fig. 3). Since the TYP offspring may have been sired by any of the males mated with the same female, it is sensible to remove them from the analyses. Though this procedere may be disadvantageous on the number of offspring for the last male in calculations, it allows to prove the last male sperm precedence. Accordingly, evaluated number of the offspring fathered by the last male might have been even higher than the actual calculations, because it seems very likely that sperm displacement mechanism has operated in *P. spumarius*. The mean numbers of the offspring (pooled data) were 10.00±2.82 and 28.12±5.05, sired by the first and the last males respectively (Fig. 3). Statistical test indicated that offspring number of the last males (74%) in the progeny was significantly higher than the first males (26%) (Mann-Whitney *U*-test, $P<0.01$). Obviously, in *P. spumarius* the last males took advantage fathering more offspring than the first males mated with females.

The mean number of the progeny produced by the multiply mated females was 29.48±6.30 in the 25 crosses. Although mean number of the progeny showed variation between the three groups (Fig. 4), average number of the progeny did not show significant difference between these groups (Kruskal-Wallis, $H=3.00$, df=2, $P>0.05$).

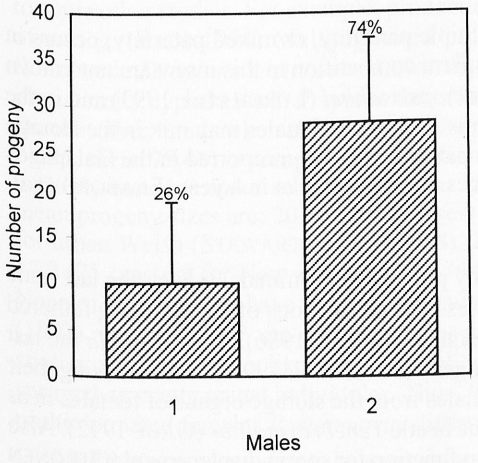

Fig. 3. Predicted number of progeny (Mean±SE) sired by the first and last males in 8 crosses, in which females mated with two different males (1: sired by first male, 2: sired by last male. (Data derived from Table II).

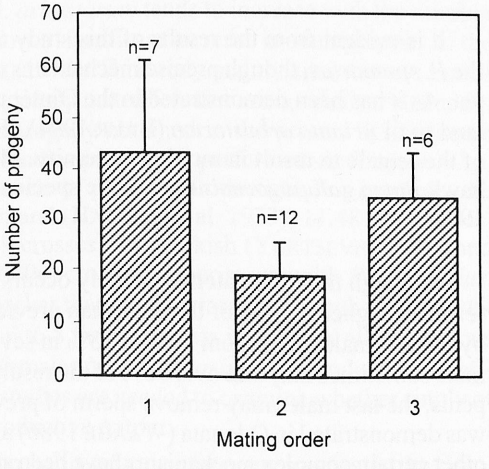

Fig. 4. Total number of progeny (Mean±SE) produced by females of different mating orders (1: mated twice and three times, 2: mated four times, 3: mated five and six times. (Data derived from Table I).

Copulation duration of individual pairs showed variation; individual females mated from 1 hour up to 56 hours with a male. Total, i.e., summarised, copulation durations of the multiply mated females ranged from 6 to 63 hours (29.00±3.34). There was a tendency for number of progeny to decline with the longer copulation duration (Fig. 5) although this was not statistically significant (r= –0.292, N=25, P>0.05).

The mean copulation duration of these three groups of females also showed variation (Fig. 6). Statistical test (one-way ANOVA: $F_{(2,22)}$=P<0.01) revealed that summarized copulation durations of the females mating four times and five+six times were significantly longer than those mated twice+three times (P<0.05, Tukey's HSD).

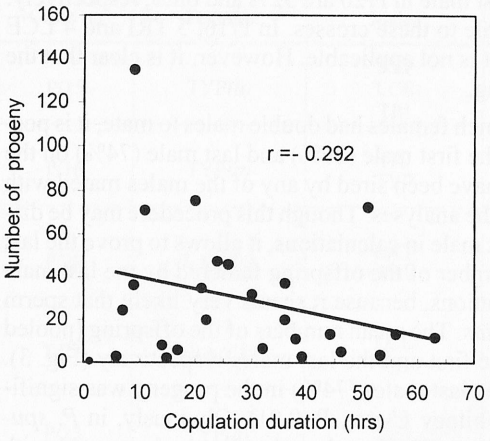

Fig. 5. Relationship between total number of progeny and total length of copulation duration (hrs) in 25 multiply mated females of *Philaenus spumarius* (N=25).

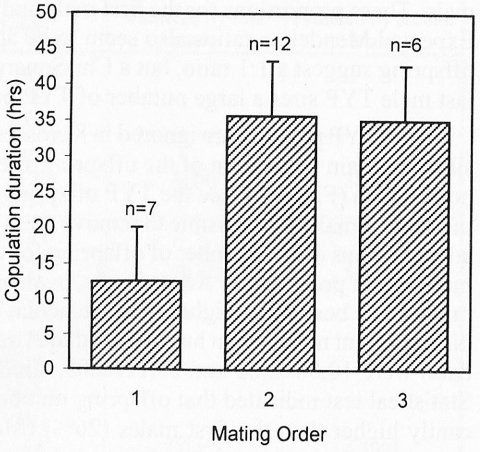

Fig. 6. Total copulation duration (hrs) (Mean±SE) by mating order of multiply mated 25 females in *Philaenus spumarius* (1: mated twice and three times, 2: mated four times, 3: mated five and six times).

IV. DISCUSSION

It is evident from the results of this study that multiple paternity, or mixed paternity, occurs in the *P. spumarius*, though precise mechanisms of the sperm competition in this insect are not known yet. As it has been demonstrated in the Dipteran *Cyrtodiopsis whitei* (LORCH et al. 1993) and in the land snail *Arianta arbustorum* (BAUR 1994), the sperms from several males may mix in the storage of the female to result in multiple paternity. Multiple paternity has been reported in the Galapagos hawk *Buteo galapagoensis* though the species produces only two chicks in a year (FAABORG et al. 1995).

Although multiple paternity widely occurs in many polyandrous animals, in fact, the last male fertilises higher portion of the eggs than previous males. The proportions of the offspring fathered by the last male range from 71% to 93% in several Odonata (WAAGE 1986). In *Drosophila* the last male sometimes may sire over 96% of the resultant progeny (PRICE et al. 1999), because, using their penis, the last males may remove sperm of previous males from the storage organs of females in as was demonstrated in Odonata (WAAGE 1986) and in the beetle *Tenebrio molitor* (GAGE 1992). Also other certain complex mechanisms have been shown to function for sperm displacement (OTRONEN & SIVA-JOTHY 1991; SIMMONS et al. 1999). A recent report indicates that the last males in *Drosophila melanogaster* may both physically displace and inhibit the use of stored sperms from previous matings (PRICE et al. 1999). Multiple paternity resulted in last male advantage in *P. spumarius*,

which is consistent with the studies discussed above. Nevertheless, the extent of the last male precedence is unclear and may be due to some sperm displacement mechanism as was stated previously.

The last male precedence is most common in many polyandrous species, though it is not a rule. For example, the first male may fertilise all eggs in the polyandrous spider *Frontinella pyramitela* (AUSTAD 1982). The first male sperm precedence seems exceptionally common in spiders (WATSON 1993) but also occurs in other polyandrous species (e.g. STILLE et al. 1986). The mating plug of males, which prevents the access of other males to mating, has been demonstrated in the butterfly *Euphydryas chalcedona* (DICKINSON & RUTOWSKI 1989) as a reason for the first male priority. Males deposit these plugs after they transferred their sperms, which make a second mating impossible (BAER & SCHMID-HEMPEL 1999). Despite the presence of the mating plugs in the garter snake *Thamnophis sirtalis*, the litters may be multiply sired (SCHWARTZ et al. 1989), because a female can store and use sperms from several males over the years (STILLE et al. 1986). However, *T. sirtalis* seems to be a very special example.

Present results do not suggest presence of mating plugs in males of *P. spumarius*. In this species mixed paternity and last male precedence have been demonstrated and estimated last male precedence may reach up to 91% (see Table II).

Copulation duration varies remarkably in many animals and may take a few seconds as in the fly *Cyrtodiopsis whitei* (LORCH et al. 1993) or minutes as in the flour beetle *Tribolium castaneum* (LEWIS & AUSTAD 1994) and Heteropteran *Lygaeus equestris* (SILLEN-TULLBERG 1981). It may even last several days as in the soappberry bug *Jadera haematoloma* (CARROLL 1991). The adaptive value of longer copulations is not clear and may be related to male fitness (WEDELL 1998; PARKER et al. 1999); it is usually associated with serious costs (e.g. increased predation) to copulating pairs (WALKER 1980; WATSON 1993). In fact, short or long copulations do not greatly affect reproductive output of the females. This was also true for *P. spumarius*. There was no significant correlation between the total copulation duration and the number of offspring, even though total duration of the copulations increase with larger number of copulations.

Hence, long lasting copulations did not give rise to increased progeny size in *P. spumarius* though there was great variation among the individual females in the duration of copulation and for interval time between the copulations. The details of the copulation duration and time intervals between the copulations on the reproductive output of the individual females and males will be published elsewhere. Therefore, these two aspects are not discussed in details in the present paper.

The number of progeny in *P. spumarius* did not increase with the number of matings, in contrast to some other studies. For example, production in *A. arbustorum* tends to increase with the number of matings (BAUR 1994). Multiply mated females show a significant increase in adult progeny of the flour beetle *Tribolium castaneum* (LEWIS & AUSTAD 1994). This study mainly aimed to test multiple paternity hypotheses in *P. spumarius*. Therefore, singly mated females are not dealt with herein. On the other hand, when the mean number of the progeny obtained in the present study (range 2-135, mean 29.48) is compared to some breeding studies previously performed on *P. spumarius*, multiply mated females seem to maintain higher levels of progeny production. For example, the mean progeny sizes are: 20.15 (209 crosses) for Finland (HALKKA et al. 1973), 11.48 (56 crosses) for urban Welsh (STEWART & LEES 1988), 27.4 (19 crosses), for Turkish (YURTSEVER 1999) and 17.2 (25 crosses) for New Zealand (YURTSEVER 2000c) crossing experiments with *P. spumarius*. None of those studies have reported a family size higher than present results. Though YURTSEVER (1997) reported that *P. spumarius* females mated up to 8 times, no data is given in other previous studies cited above. It is likely that multiply mated females of *P. spumarius* may produce more offspring than singly mated individulas. Thus, further studies are needed to examine whether singly or multiply mated females *P. spumarius* differ in reproductive output.

Multiple mating appears to be a requisite and is probably adaptive for many species. In the Colorado potato beetle *Leptinotarsa decemlineata* at least 3 matings are required to fill the female's spermatheca (BOITEAU 1988). One insemination is not enough to fill spermatheca of a virgin female in the damselfly *Coenagrion scitulum* (CORDERO et al. 1995). Thus, the female fertility is associated

with multiple mating. Multiple mating enhances fertility, because there might be inadequate number of sperm for fertilisation from the first mating due to sperm depletion (YAMAGISHI et al. 1992) or proportions of matings might be impotent (RIDLEY 1988). Number of sperms stored in the female leafcutter ant *Atta colombica* is positively correlated with the number of mates and enhanced female fitness (FJERDINGSTAD & BOOSMA 1998).

Multiple mating may provide many advantages to polyandrous species, because females gain increased fitness through male contributed nutrients. For example, FRIEDEL and GILLOT (1977) showed that nutritious male secretions derived from the seminal fluid might be absorbed in the genital tract, resulting in enhanced fecundity in the females. In many Lepidoptera species, nutritious substances of spermatophores increase reproductive output and longevity of the females, and the mass of these contents is increased with degree of polyandry (SWARD & WIKLUND 1989; BISSOONDATH & WIKLUND 1995). Therefore polyandrous species will have substantially higher lifetime and fecundity compared to monoandrous species (RIDLEY 1988).

Multiple mating may provide significant evolutionary benefits to polyandrous species through increased genetic heterogeneity, though it is debated that multiple mating does not contribute greatly to the genetic diversity (TAYLOR 1967; WADE 1982; YASUI 1998). BAER & SCHMID-HEMPEL (1999) working on a bumble-bee (*Bombus terrestris*) examined artificially inseminated queens corresponding to single mating and multiple mating, and found that multiple mating did not increase the colony size (as in the present study), but it increased genetic diversity within the colony. Genetically diverse colonies exhibited better resistance to the parasites, and the high diversity colonies had higher reproductive success through polyandry. Hence, multiple mating with different partners enhances genetic variety within a brood (BAUR 1994; REICHARDT & WHEELER, 1996) and may provide maximal genetic diversity (LEVINE et al. 1980) with increased female fitness (LOMAN et al. 1988; RIDLEY 1988; MADSEN et al. 1992).

Although the incidence of multiple mating and multiple paternity in natural populations of the meadow spittlebug *P. spumarius* is not well known, there is evidence that they occur in the wild (YURTSEVER 1997). However, it remains to be seen how common multiple mating behaviour in *P. spumarius* is in natural populations. The extent of this behaviour and the mechanism ensuring the last sperm precedence are also unclear and they have yet to be investigated. The major evolutionary consequence of polyandry in *P. spumarius* may be genetically diverse progeny if it widely occurs in the natural populations, because the progeny of a female which mated with different males will positively include more heterozygous individuals than singly mated females. Since this homopteran insect shows very high habitat diversity (STEWART & LEES 1996; YURTSEVER 2001), multiple mating and multiple paternity may be some of the factors influencing the meadow spitlebug's wide global distribution through increased genetic variability and high fitness.

In conclusion, the present study associated with colour pattern polymorphism is the first evidence of multiple paternity in *P. spumarius*. The results reveal that this homopteran insect and its polymorphism provide valuable material for the study of sperm competition and its possible consequences as they offer the opportunity to study evolution in action.

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