LEG SIZE AFFECTS MALE MATING SUCCESS IN *TARSONEMUS CONFUSUS* EWING (PROSTIGMATA: TARSONEMIDAE)

BY N. GARGA *, H. PROCTOR *[1] and R. BELCZEWSKI *

MORPHOLOGY
TARSONEMUS CONFUSUS
MATING
AGGRESSION

SUMMARY: Legs IV of males in the family Tarsonemidae are highly modified to form thickened pincer-like appendages. Previous literature describes males using legs IV both to manipulate pharate females onto the males' genital capsule as well as to constrain the adult female's legs during mating. We observed behavioural interactions between males and females from three populations of *Tarsonemus confusus* Ewing. We did not witness males using legs IV in the reported manner, but rather saw them using legs IV in contests for possession of pharate females. Male body size and leg IV size were positively correlated for the three populations of *T. confusus*. However, the correlation was imperfect and males of the same body size often had different leg sizes. When two males were placed into an arena with a pharate female, the male that succeeded in capturing and guarding the female had legs IV that were significantly longer and larger (by area) than those of the loser. However, there was no significant difference in body size between the successful and unsuccessful males.

MORPHOLOGIE
TARSONEMUS CONFUSUS
ACCOUPLEMENT
AGRESSION

RÉSUMÉ : Les pattes IV des mâles de Tarsonemidae sont fortement modifiées, formant des appendices épaissis semblables à des pinces. Des exemples de mâles utilisant les pattes IV à la fois pour manipuler des femelles sur la capsule génitale mâle et aussi pour retenir les pattes des femelles adultes pendant l'accouplement ont été cités dans la littérature. Nous avons observé des comportements interactifs entre les mâles et les femelles dans trois populations de *Tarsonemus confusus* Ewing. Nous avons vu des mâles utiliser leurs pattes IV dans une compétition pour la possession des femelles plutôt qu'utiliser leurs pattes IV de la manière rapportée. Nous avons obtenu une corrélation positive entre la dimension corporelle des mâles et la taille des pattes IV dans trois populations de *T. confusus*. Par contre, la corrélation était imparfaite chez les mâles d'une dimension corporelle identique qui possédaient fréquemment des pattes de tailles différentes. De deux mâles placés dans une arène avec une femelle pharate, le mâle qui a réussi à saisir et garder la femelle avait des pattes IV considérablement plus longues et plus larges que celles du perdant. Il n'y avait cependant pas de différence significative entre les dimensions des mâles gagnants ou perdants.

* Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.
1. Present address (to which reprint requests should be addressed): School of Environmental Studies, Griffith University, Nathan 4111, Queensland, Australia.

Introduction

The Tarsonemidae is one of many mite families that exhibit sexual differences in leg morphology. In this group, legs IV of males are thickened and terminate in sessile claws, while in females they are greatly reduced and end in long whip-like setae (Lindquist, 1986). In a behaviour known as precopulation, male tarsonemids pick up pharate females and attach them to a dorsally oriented adhesive organ called the genital capsule. The female remains with the male for up to 24 hours until she ecloses as an adult. Mating occurs as soon as the female frees herself of the exuviae. It has been suggested that male legs IV are used both during the initial phase of precopulation, to grasp a pharate female and place her on the male's genital capsule, as well as during copulation, to pin legs IV of the female (Karl, 1965, Lindquist, 1986). There are innumerable other examples of legs modified for holding females while mating in both the Prostigmata (e.g. Proctor, 1992) and the Mesostigmata (e.g. Faasch, 1967). However, modified legs in male mites are not always used solely to grasp females, as they are sometimes employed in inter-male aggression [e.g. *Macrocheles muscaedomesticae* (Scopoli) (Yasui, 1988); many species in the Acaridae (Radwan, 1995)].

To elucidate the role of modified legs IV in male tarsonemids more fully, we made extensive observations of precopulatory behaviour in *Tarsonemus confusus* Ewing when more than one male was present. As well, we investigated the relationships between leg IV size and body size, and determined whether either of these measures was a good predictor of male mating success.

Materials and methods

Mite collection

Tarsonemus confusus Ewing is a small, fungivorous mite found on the leaves of many plants, including bananas, dahlias, strawberries and irises (Ewing, 1939; Lindquist, 1986). There are four easily recognizable stages in the life cycle: egg, larva, pharate nymph and adult male and female (Lindquist, 1986).

Experimental populations of *Tarsonemus confusus* were collected from three different sites. The Lab Stock population was obtained from the Biology Department at Queen's University, Kingston, Ontario. These mites were originally collected from leaves in an apple orchard at the Agricultural Canada experimental farm in Smithfield, Ontario, in the autumn of 1993 and had spent a year under laboratory conditions. They were maintained on *Cladosporium* sp. fungi grown on 2% malt agar at 26°C with a light:dark cycle of 18:6 h. The Opinicon population was collected from apple leaves at the Queen's University Biological Station near Chaffey's Locks, Ontario in September 1994. The third population, Smithfield, was also collected in September 1994, from the same orchard as the Lab Stock. The latter two populations were maintained under the same conditions as Lab Stock. The three populations were kept in separate cultures and no interbreeding occurred.

Laboratory culture

Mites were maintained in plastic petri dishes containing 2% malt agar medium. A ring of Tangle Foot© was applied to the inside edge of the petri dish to prevent mites from crawling out. On top of the agar, we placed a round sheet of sterilized polyvinyl with three small circles cut out (diameter *ca* 4mm). A drop of sterilized distilled water mixed with *Cladosporium* sp. hyphae and spores was placed into each of the three circles. This resulted in fungus growth through the excised circle, which made observing the mites much easier. The polyvinyl was also beneficial because mites have higher survivorship on the polyvinyl as compared to the agar surface (personal observation R.B.).

Experimental design

Experiments and measurements were carried out from September 1994 to January 1995. We designed our experiments with the *a priori* hypothesis that male body size would be related to mating success. Using a dissecting microscope, we chose a male with a large body size and a male with a smaller body size and placed both in a new culture dish. Males from different populations were sometimes paired in these experiments. We removed a pharate female from the back of a third male and introduced her to the petri dish with the two males. The male originally carrying the female was not used in the experiment. The dishes were checked every 10–15 minutes under a dissecting microscope to see if the pharate female had been picked up. Once a male had acquired a pharate female, we made more frequent checks to observe interactions between the two males. If there was no confrontation within an hour of one male picking up the female, or if the adult female moulted and mating occurred, we removed both males from the dish. If a confrontation occurred, we observed the males for a longer period of time to be certain of which male acquired possession of the pharate female. After a trial, males were mounted individually in Hoyer's medium and labelled as being with or without the pharate female at the trial's termination.

Mite measurements

Males from the three populations were measured to determine the correlation between leg size and body area. We sampled 20–22 males from each population and mounted them in Hoyer's medium. Slides were examined under a compound microscope at 200 ×. A Panasonic camera attached to the microscope transferred the image to a video monitor where we drew an outline of the mite on an acetate placed directly on the monitor. The outline on the acetate was digitized and calibrated using a Graphics Master (Numonics™) digitizing tablet and the Sigma Scan (Jandel Scientific™) program. We took three different measurements from each acetate: body area, leg IV area and leg IV length. The left leg was consistently chosen for leg IV measurements. Data were analyzed using Excel 5.0 (Microsoft© Corporation). By repeating each measurement three times we determined that the error of our measuring system was ± 5 µm. Males from the mating trials were also measured in this way. Measurements were compared using ANOVA for populations and paired t-tests for mating trials.

Results

Mating Behaviour

Current literature states that the fourth legs of tarsonemid males are used to help move the female onto the male's back during precopulation and to hold or pin the female's legs III and IV during copulation (Nucifora, 1963; Karl, 1965; Lindquist, 1986). Although we watched over 50 copulations, we neither observed male *T. confusus* using legs IV to hold the female's legs during copulation, nor did we witness them grasping and moving the pharate female with legs IV. However, we could not exclude their possible use in positioning the pharate female during precopulation because males slid their legs IV under the pharate females prior to precopulation, thus obscuring our view.

In six of approximately 100 casual and experimental observations, we observed males fighting each other end to end with their fourth legs. Upon encountering a precopulatory pair, the intruding male climbed onto the pair and tapped them with his first legs. The intruder then used his legs IV to snip at the adhesive margins of the other male's genital capsule and slid them beneath the body of the pharate female. At this point a tug-of-war ensued in which each male faced a different direction and tried to pull the pharate female from the grip of the other male. Karl (1965) also observed more than one male *T. confusus* attempting to capture a single pharate female. In our observations it was unclear whether the males maintained their grip on the female purely via the adhesive properties of their genital capsules, or whether they also used their legs IV to pull. The most impressive multi-male encounter we observed commenced with one male guarding a pharate female while four other males fought for possession. The pharate female was moved around between all the males throughout the encounter, which lasted approximately 45 minutes, before the female was accidentally swept off the back of one male by fungal hyphae. The male who was last guarding her did not come back for her nor did any other male.

We observed that adult males would occasionally pick up pharate instars that developed into adult

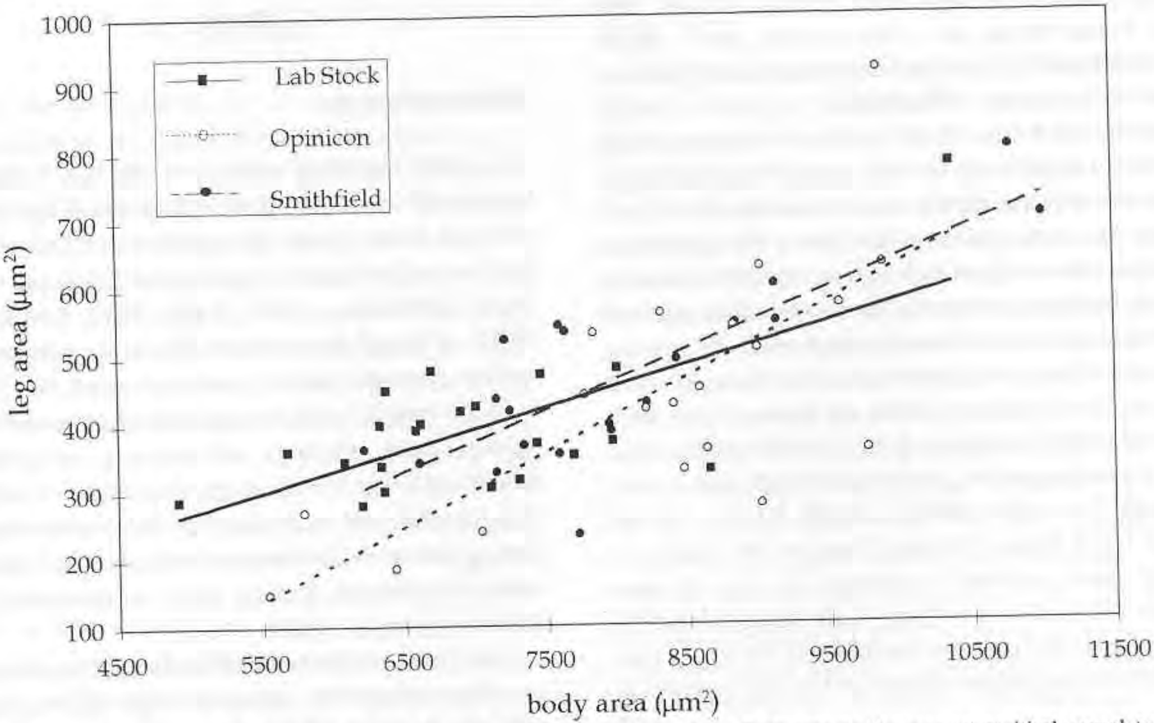

FIG. 1: Relationship between body area and leg area for three populations of *T. confusus*. Body area and leg area are positively correlated (Lab Stock, $r^2 = 0.4343$, $p<0.01$, $y = 0.6278x - 0.0004$, $n = 22$, Opinicon, $r^2 = 0.5592$, $p<0.01$, $y = 0.1097x - 0.0005$, $n = 20$, Smithfield, $r^2 = 0.6514$, $p<0.01$, $y = 0.0887x - 0.0002$, $n = 20$).

males. This occurred nine times in the forty instances in which the sex of the pharate was monitored. KARL (1965) also observed homosexual pairings in *T. confusus*.

Correlation between body area and leg size

The average body area for the three populations was 7000 ± 240 µm² for Lab Stock, 8390 ± 270 µm² for Smithfield and 8050 ± 260 µm² for Opinicon (Fig. 1). Lab Stock population was significantly smaller in body area than mites from Smithfield and Opinicon (ANOVA, $F = 7.559$, $p = 0.0012$, $n = 63$, Tukey HSD, $p = 0.021$ and $p = 0.001$ respectively). Smithfield and Opinicon populations did not differ from each other (Tukey HSD, $p = 0.628$). There were strong correlations in all three populations between body area and the leg size as measured by leg area (Fig. 1). It is important to note, however that the correlations were not perfect; males sharing the same body size could differ greatly in leg size. There were no significant differences among the leg/body correlation coefficients of the three populations ($\chi^2_{exp.} = 1.018$, $\chi^2_{.05[2]} = 0.991$, $p>0.05$).

Male success and leg size

Fourth legs of males that successfully secured pharate females were larger in area then those of unsuccessful males (paired t-test on left leg area, sequential Bonferroni adjusted, $t = -3.8513$, $p<0.01$, $n = 24$, Fig. 2, Table 1). Legs were also longer in males with pharate females than males without them (paired t-test, sequential Bonferroni adjusted, $t = -2.1296$,

	males with pharate (± S.E.)	males without pharate (± S.E.)	*P*
Body area (µm²)	60.70 (233)	61.30 (195)	0.4092
Leg length (µm)	46.20 (4.31)	37.22 (0.77)	0.0022
Leg area (µm²)	3.940 (0.18)	3.150 (0.18)	0.0004

TABLE 1: Mean measurements (± standard error) taken from *T. confusus* males paired against each other in competition for possession of a pharate female (n = 24 pairs).

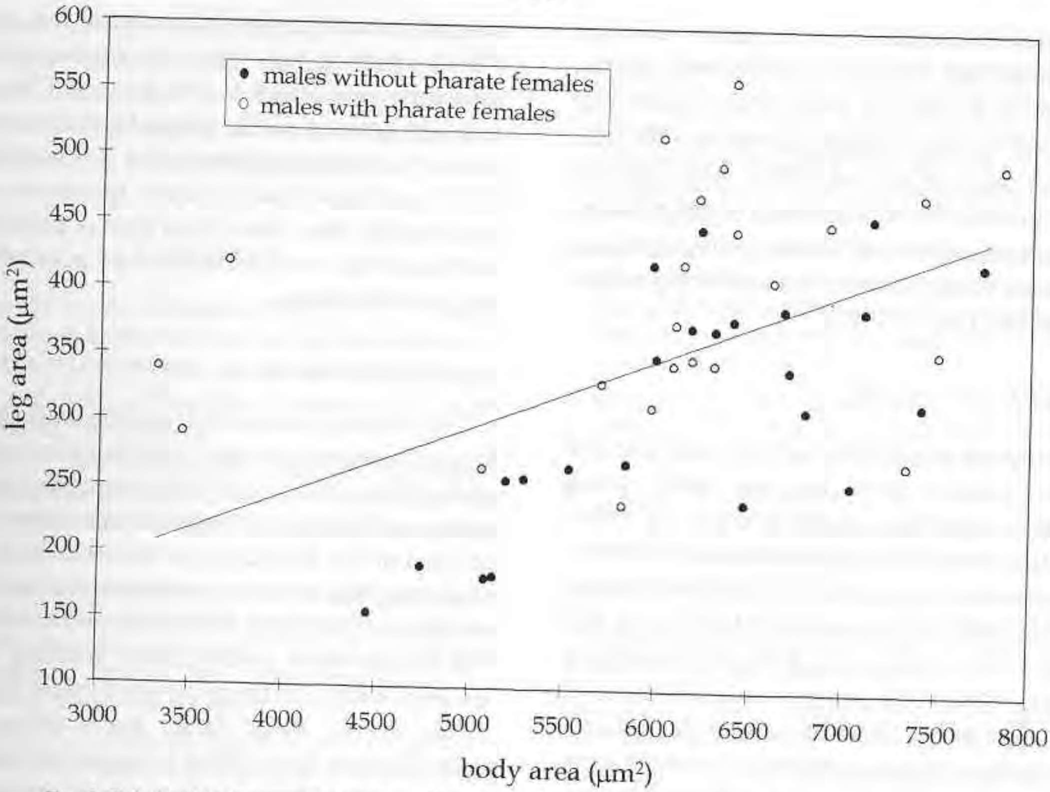

FIG. 2: Relationship between body area and leg area of male *T. confusus* with and without pharate females.

$p<0.01$, $n = 24$, Table 1). However, no difference was found between the body area of males with and without pharate females (paired t-test, $t = 0.2321$, sequential Bonferroni adjusted, $p>0.41$, $n = 24$, Table 1).

DISCUSSION

Role of fourth legs in male-male competition in Tarsonemus confusus

The few publications pertaining to tarsonemid mating behaviour state that legs IV of males function to lift the pharate female and place her on the male's posterior idiosoma, where she is held by the genital capsule (NUCIFORA, 1963; KARL, 1965; LINDQUIST, 1986). LINDQUIST (1986) also cites observations suggesting that the male's third and fourth legs may be used to pin or block legs III and IV of the female during copulation. NUCIFORA (1963) illustrated the blocking action of male legs III and IV in *Polyphagotarsonemus latus* (Banks). In contrast, we did not observe pinning during copulation, but rather found that male *T. confusus* used legs IV aggressively to remove pharate females from the possession of other males. Although only six of our 100 observations involved fighting, the frequency of fights was likely greater. Our six observations were chance sightings, as we did not continuously observe use of legs IV during experimental trials. We may also have missed some interactions when fungal hyphae obscured the view. Although KARL (1965) also noted that two males of *T. confusus* occasionally "grabbed" a single pharate female, the precise way that legs IV operate in male-male struggles was not clear; however, because the legs are thickened in a way that suggests hypertrophied musculature, they may be used to exert force against the suction grip of their rival's genital capsule in order to wrest the pharate female away.

The observation that male *T. confusus* use their legs in aggressive interactions is not unique to this species. Male *Macrocheles muscaedomesticae* (Scopoli) (Macrochelidae) guard pharate deutonymphal females and when challenged by other males use their heavily spurred legs III and IV as weapons (YASUI, 1988). Fighter morphs of male acarid mites have talon-like legs III that are used to puncture the integuments of rivals (RADWAN, 1995). Although not obviously modified for combat, legs I of male *Schizotetranychus miscanthi* Saitō (Tetranychidae) are significantly longer in populations that exhibit high intermale aggression (SAITŌ, 1995).

Morphometric relationships

The continuous relationship between body size and leg size in *T. confusus* males indicates that they lack distinct leg morphs based on body size. This differs from the classic situation in horned beetles (Tenebrionidae, Scarabeidae, Lucanidae) in which two distinct male morphs exist: large males with large horns and small males with small or no horns (EBERHARD & GUTÍERREZ, 1991). The continuous distribution of leg size in *T. confusus* also differs from the dichotomous situation in many acarid mites, in which there are large-legged, aggressive males and small-legged, passive males (RADWAN, 1995).

Although leg size was positively correlated with body size in *T. confusus*, the correlation was imperfect (Fig. 1), and small-bodied males often had larger legs IV than did much larger-bodied males. Extreme variation in leg IV size has been documented in males of other species of *Tarsonemus: T. nodosus* Schaarschmidt (KALISZEWSKI *et al.*, 1983) and *T. waitei* Banks (LINDQUIST, 1978). In fact, KALISZEWSKI *et al.* (1983) found that leg IV size was the most variable of 132 characters they measured in male and female *T. nodosus.*

The difference in body size between Lab Stock mites and those from Smithfield and Opinicon populations may result from their having spent different times in captivity. Opinicon and Smithfield mites were both removed from the field in September 1994 and had spent only a few weeks under laboratory conditions before being sampled. Lab Stock, however, had spent one year in a laboratory environment prior to being sampled. The laboratory environment differs from the natural environment in temperature, humidity, presence of predators and competitors, sex ratios and types of food, and may have caused or selected for smaller size. In support of this, one of us (N.G.) observed that when the *Cladosporium* food source became infected with bacteria (Dec. 1994), mites maintained on this fungus became increasingly smaller in subsequent generations. KALISZEWSKI *et al.* (1983) also found that lab-reared *Tarsonemus nodosus* were smaller than those from field populations and attributed this to a combination of poor laboratory diet and inbreeding.

Male size and mating success

The discovery that male *T. confusus* with larger and longer fourth legs are able to outcompete conspecifics with smaller fourth legs is not surprising given that males use their legs to wrestle females from the grasp of other males. Body area, on the other hand, did not play a significant role in determining the winner of an encounter. This is surprising contrast to other taxa in which males show precopulatory guarding of females. For example, in the twospotted spider mite *Tetranychus urticae* Koch, larger males defeat smaller males (POTTER *et al.*, 1976). In the isopod crustacean *Iodotea baltica* (Pallas), takeovers occurred in 69% of the cases in which the resident male was smaller and in 14% when the resident male was larger (JORMALAINEN *et al.*, 1994).

In *T. confusus* the trait that seems to increase a male's mating success is the size of legs IV. If one of the most important factors for securing females in *T. confusus* is amount of pressure exerted by the tips of leg IV, then larger legs may have more room for internal musculature irrespective of overall body size. Our results show that relative leg IV size is a good predictor of potential mating success of a male *T. confusus*; similarly, leg I length of male *Schizotetranychus miscanthi* is a variable that frequently predicts the winner of male-male combat in this species of spider mite (SAITŌ, 1995). It may be that modified male legs in many other mite taxa are also the result of selection for greater prowess in intrasexual combat.

ACKNOWLEDGEMENTS

We would like to thank Dr D. HARMSEN for his help in designing the project, Dr E. LINDQUIST for providing literature on tarsonemids and Dr D. WALTER for his helpful comments on an earlier draft of this manuscript. This project was funded by a Natural Science and Engineering Research Council of Canada Operating Grant awarded to H.P.

REFERENCES

EBERHARD (W. G.) & GUTÍERREZ (E. E.), 1991. — Male dimorphism in beetles and earwigs and the question of developmental constraints. — Evolution, **45**: 18-28.

EVANS (G. O.), 1992. — Principles of Acarology. — C.A.B. International, Oxon. 563 pp.

EWING (H. E.), 1939. — A revision of the subfamily Tarsoneminae of North America, the West Indies, and the Hawaiian Islands. — Tech. Bull. U. S. Dept. Agric., **653**: 1-63.

FAASCH (H.), 1967. — Beitrag zur Biologie der einheimischen Uropodiden *Uroobovella marginata* (C. L. Koch 1839) und *Uropoda orbicularis* (O. F. Müller 1776) und experimentelle Analyse ihres Phoresieverhaltens. — Zool. Jahrb. (Syst.), **94**: 521-608.

JORMALAINEN (V.), MERILAITA (S.) & TUOMI (J.), 1994. — Male choice and male-male competition in *Idotea baltica* (Crustacea, Isopoda). — Ethology, **96**: 46-57.

KALISZEWSKI (M.), SUSKI (W.) & REJMAN (S.), 1983. — Individual variation in wild and laboratory reared specimens of *Tarsonemus nodosus* Schaaeschmidt, 1959 (Acari: Tarsonemidae). — Folia Ent. Hung., **44**: 63-81.

KARL (E.), 1965. — Untersuchungen zur morphologie und Ökologie von Tarsonemiden gartnerischer kulturpflanzen. II. *Hemitarsonemus latus* (Banks), *Tarsonemus confusus* Ewing, *T. talpae* Schaarschmidt, *T. setifer* Ewing, *T. smithi* Ewing und *Tarsonemoides belemnitoides* Weis-Fogh. — Biol. Zentralbl., **84**: 331-357.

LINDQUIST (E. E.), 1978. — On the synonymy of *Tarsonemus waitei* Banks, *T. setifer* Edwing, and *T. bakeri* Ewing, with redescriptions of species (Acari: Tarsonemidae). — Can. Entomol., **110**: 1023-1046.

LINDQUIST (E. E.), 1986. — The world genera of Tarsonemidae (Acari: Heterostigmata): a morphological, phylogenetic and systematic revision with a reclassification of family-group taxa in the Heterostigmata. — Mem. entomol. Soc. Can., **136**: 517 pp.

NUCIFORA (A.), 1963. — Osservazioni sulla riproduzione di *Hemitarsonemus latus* (Banks) (Acarina: Tarsonemidae). — Atti Acad. Naz. Ital. Entomol. Rend., **10**: 142-153.

POTTER (D. A.), WRENSCH (D. L.) & JOHNSTON (D. E.), 1976. — Guarding, aggressive behaviour, and mating success in male twospotted spider mites. — Ann. entomol. Soc. Amer., **69**: 707-711.

PROCTOR (H. C.), 1992. — Mating and spermatophore morphology of water mites (Acari: Parasitengona). — Zool. J. Linn. Soc., **106**: 341-384.

RADWAN (J.), 1995. — Male morph determination in two species of acarid mites. — Heredity, **74**: 669-773.

SAITŌ (Y.), 1995. — Clinal variation in male-to-male antagonism and weaponry in a subsocial mite. — Evolution, **49**: 413-417.

YASUI (Y.), 1988. — Sperm competition of *Macrocheles muscaedomesticae* (Scopoli) (Acarina: Mesostigmata: Macrochelidae), with special reference to precopulatory mate guarding behaviour. — J. Ethol., **6**: 83-90.