A review of *Nasonia* (Chalcidoidea, Pteromalidae) courtship and mating behaviour, with some additional, new observations

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*Nasonia* is emerging as a model organism for several biological disciplines. We describe the essential aspects of courtship and mating behaviour in the three known *Nasonia* species. All three species share a basic pattern but can be distinguished on qualitative and quantitative differences. The possibility to produce hybrids opens up the study of the genetic architecture of courtship and mating behaviour.

**Keywords:** behavioural genetics, courtship, *Nasonia*

**NASONIA** as experimental animals

Over the last decennia, *Nasonia* (ca. 2-3 mm long, parasitic wasps) have served successfully as experimental animals in several branches of biological research (Beukeboom & Desplan 2003, Pultz & Leaf 2003, Shuker *et al.* 2003). These include quantitative genetics of populations (Beukeboom & Werren 1992, Orzack & Gladstone 1994); sex ratio distorters (Nur *et al.* 1988); genomic-cytoplasmic interactions (Breeuwer & Werren 1993, 1995); analysis of courtship and mating behaviour (Van den Assem 1986, 1996); genetics of courtship behaviour (Beukeboom & Van den Assem 2001); bacteria-induced post-mating incompatibility (Breeuwer & Werren 1990, Bordenstein *et al.* 2001); and genome mapping of several traits (Gadau *et al.* 1999). The widespread use need not surprise in view of *Nasonia*’s attractive practical advantages in the laboratory, such as: (1) Maintenance of cultures is simple. (2) Cultures take up little space. (3) Practically any number of specimens can be made available at short notice. (4) Productions are low-cost. (5) Specimens can be sexed before they eclose from their pupae. (6) Successive generations emerge at 2-week intervals (at 25°C). (7) *Nasonia* parasitize the pupae of cyclorrhaphous flies. Blowfly maggots are grown commercially and are widely available. Hence, no need for parallel cultures of hosts. (8) *Nasonia* males are flightless or nearly so; females do not immediately fly away, making etherisation prior to handling unnecessary. (9) Larvae can be induced to enter into diapause for a one-year period or longer. This will preserve a strain’s genetic make-up over extensive periods. In cultures with a high turnover of generations, effects of undesired selection will appear sooner or later (Van den Assem & Jachmann 1999). (10) *Nasonia* can be collected in the field: parasitized hosts from bird nests or nest boxes (after the young have left), or around carcasses; ovipositing females on exposed bait (a small quantity of smelly meat with a few host pupae in a small gauze bag pinned onto a tree trunk, sheltered from rain and, as far as possible, ants). (11) As all Hymenoptera, *Nasonia* have haplo-diploid sex-determination, which is an asset in genetic research.

**THE STUDY OF COURTSHIP AND MATING BEHAVIOUR**

Our present topic of interest is courtship and mating behaviour: description, causal analysis, effects and function, and comparison. Accurate, quantitative descriptions of motor patterns and repertoires are basic to all further steps. Without reliable descriptions, studies with a focus on underlying causal factors, which are responsible for the temporal organisation of displays, are not well possible, nor are those focussing on effects, *i.e.* on identification of those stimuli that induce sexual receptivity in virgin conspecific females in the first place. The comparison of displays of related species can provide indicators of evolutionary gradients (Hölldobler & Wilson 1983) – hence
provide an insight in more ancestral and more advanced conditions. Results may contribute to more encompassing questions, such as the role of courtship in the maintenance of species integrity on the one hand, and its role in the genesis of species diversity on the other. A number of chalcidoid species has been used in studies of this kind already, those of *Nasonia* not in the last place.

Three species of *Nasonia* are known: the widespread *N. vitripennis* (Walker 1836), and two others: *N. giraulti* and *N. longicornis* (Darling & Werren 1990), restricted to North America, with allopatric distributions, but sympatric with *vitripennis*. *Nasonia* courtship and mating behaviour have been described and discussed before; for *vitripennis* see e.g. Cousin (1933), Barrass (1960a,b, 1961), Whiting (1967); for all three species: Van den Assem & Werren (1994). The respective displays are sufficiently diverse to ensure a reliable identification on the species level; differences are both qualitative (species-characteristic motor patterns) and quantitative (number and/or duration of similar elements).

Heterospecific matings may occur but rarely result in viable offspring. The post-mating isolation is maintained by mutually incompatible strains of endosymbiotic *Wolbachia* bacteria (Breeuwer & Werren 1990), which can be removed by feeding antibiotics (Bordenstein & Werren 1998, Bordenstein et al. 2001). Crosses of so-called ‘cured’ lines can produce hybrid offspring. Genetic differences between *Nasonia* species have been demonstrated before (Breeuwer & Werren 1995); hence, the study of hybrid displays (Beukeboom & Van den Assem 2001) could contribute to insights in the genetics of courtship behaviour. Hybrid courtship can also be used in studies of reproduction-isolating factors (Coyne 1992).

**GENERAL ASPECTS OF NASONIA DISPLAYS**

In general, mating in insects does not come about by chance but potential partners must be located and ‘pacified’. Next, females have to be made receptive, which requires stimulation on the part of males. Frequently, this is provided with a performance of some kind, called courtship behaviour. *Nasonia* court in a stereotype position on top of the female, with the forefeet placed on her head (Fig. 1). Displays include highly stereotype movements with various limbs (antennae, mouthparts, wings, legs, and the entire head) that never appear at random, although an untrained eye may judge otherwise. In fact, there is a strict order.

A striking aspect of a *Nasonia* display is its cyclical nature (Fig. 2): similar combinations of elements are repeated at intervals and combinations are separated from one another by pauses of gradually increasing duration (Van den Assem 1975). Series of headnods are a conspicuous component of any *Nasonia* display cycle. Number of nods per series may change but never in an erratic way; the general trend is a moderate increase. (In fact, series of nods are exceptional among pteromalids; the large majority produce cycles with single nods).

![Figure 1. Nasonia male courts on top of female](image-url)
The display’s periodic properties do not result from external input, e.g. female ‘responses’. The simple proof is that dummy females, which represent a constant stimulus-situation throughout, are courted in the same way as life females. Hence, the cyclic appearances reflect periodic changes in the courting male’s endogenous condition, which were verified in experiments (Jachmann & Van den Assem 1993). The discharge of pheromones that provoke overt receptivity in potentially receptive females (products of mandibular glands) is a key factor in this context. Experimental evidence (Van den Assem et al. 1980a) shows that pheromone discharges relate to mouthpart extrusions, a rather inconspicuous motor pattern. In *Nasonia* and close relatives these extrusions are tied one to one to very conspicuous headnods (convenient for the observer). Interestingly, in ancestral groups extrusions are always present but headnods absent or inconspicuous wobbles at best. With these observations in mind, it is arguable that nodding represents an evolutionary more recent phenomenon. Most likely, nodding improves the effect of discharges: the mouthparts move closely along the female’s antennae, which carry numerous chemoreceptors. Experimental results do not support this supposition. Fully intact males and males with an immobilised head were equally successful, in total receptivity scores and in time required to provoke receptivity.

Wing vibrations are another part of the displays. However, wings themselves seem to be of no importance: males with wings that were fully removed or weighted did not score differently from intact males in courtship success. However, both were able to produce identical sounds, which result from vibrations of the wing musculature. The chitinous wall of the thorax seems to serve as a resonator. Courtship sounds are species-characteristic but in spite of this specificity a biological function is uncertain (Van den Assem & Putters 1980). Males can be silenced by applying a droplet of superglue to the top of the thorax. Displays of intact and mute males were equally effective.

In the same vein, parts of the antennae and tarsi can be removed. However, these interventions incur complications. Males without fore tarsi are seriously handicapped in pursuit of and mounting on females; those without antennae lose the ability of proper orientation. Less drastic interventions such as one-sided or incomplete removals had no noticeable effect on courtship success.

In the end it seems that the discharge of mandibular pheromones is the one and only display component with a straightforward biological function: provoking overt female receptivity. Chemical signals are probably the oldest means of communication in insect evolution; chemoreceptors can function with incredible precision. The results of three experiments support the pride of place of chemical means in *Nasonia* courtship. (1) Males that had their mouthparts sealed but were intact otherwise courted in a normal way without ever provoking receptivity. The
delivery of a small volume of air loaded with pheromone particles brought them immediate success (Van den Assem et al. 1980a, Van den Assem 1996). (2) Abdomectomized males court vigorously but neither extrude the mouthparts nor nod their head, perhaps due to pressure changes inside the head capsule: courted females remained unresponsive. However, sealing the male’s injury with a droplet of superglue seemed to restore the essential pressurizing mechanism: headnods and extrusions reappeared and, presumably, pheromone discharge took place again: virgin females promptly signalled receptivity (Van den Assem et al. 1981). (3) Females may show overt receptivity without being courted at all; a high enough concentration of pheromone in the surroundings will induce spontaneous receptivity (Van den Assem 1996). However, soon enough a persistently high concentration will provoke a reverse reaction: male display behaviour will come to a halt and females will immobilise. High enough concentrations are unlikely to appear in natural situations. *Nasonia* males emerge from the host puparium before females do, and court out in the open where an accumulation of volatile pheromones is unlikely to appear.

The idea that most of the species-characteristic motor patterns of displays should be void of biological function is unsatisfactory, the more so because the diversity fits in so well with expectations. It seems quite possible, however, that the various motor patterns do play an important role, but elsewhere in the process. Not in the inducement of receptivity per se, but by creating the condition in which receptivity may be induced. Courtship can only lead to success if the female remains immobile during the performance. It is in this domain that the variety of motor patterns can play a role. Probably not in a rigid way, as the fixed sum of the various contributions, but more flexible. If some part is lacking (e.g. no sounds, as in the case of mute males) other components may come to play a relatively more important role. Direct experimental evidence on this point will not be easy to obtain.

**THE END OF A DISPLAY**

*Nasonia* males do not continue to court females that fail to show receptivity within a certain period of time; dismounting will soon follow. Peripheral causes of dismounting of male origin such as ‘fatigue’ or pheromone depletion are unlikely agents. Males are able to court almost continuously and they can induce receptivity in hundreds of virgin females in a row (Barrass 1962). Depletion of the pheromone stock in the course of a single display seems therefore unrealistic. Yet, there is some doubt on this point. Usually, virgin *vitripennis* females will signal receptivity before the start of the fourth cycle; many will do with the first or second cycle. Manipulated females (i.e. females that were not immediately exposed to a post-copulatory display, see next paragraph) will show renewed receptivity, but only after many more cycles). A male will usually provide for that much; however, receptivity frequently fails to reappear and dismounting follows. Often enough, the male will remount soon after (within 5 to 10 s) on the same female and court again. In nearly all such cases receptivity appeared with the first headnod. It is unrealistic to suppose that the previous dismounting occurred just one cycle too early to gain success. A better conclusion is that the readily available pheromone supply was depleted during the previous display and that it did not immediately lead to dismounting. In a competitive situation (apparently the normal one in *Nasonia*; Van den Assem et al. 1980b) prolongation of a display in a zero-success situation seems to be a waste of time and therefore counter-productive (Parker 1978). Whatever the theoretical implications, short-term depletion of mandibular pheromones in *Nasonia* cannot be a cause of dismounting. *Nasonia* males seem to keep a limited supply of pheromones for immediate release, perhaps somewhere at the periphery, and a much larger stock in the mandibular glands from where the ready-available station is replenished, during periods off the female, or during mounting.

Female-produced external stimuli are not involved in the timing of dismounting either: displays on dummy females and on fully intact, unreceptive females were of similar duration, on average. A good argument for an endogenous control of dismounting is that the duration of a particular display depends on time since the previous display and its result (either unsuccessful or with copulation success). Males that court a series of unreceptive females in a row arrive at a minimum duration (such ‘habituated’ displays include about 2 head nod series; after all enough to induce receptivity in a majority of virgin *Nasonia*). Habituated males were exposed to an extremely low
temperature for a short period (we used -30°C during half an hour in a freezer; males were inside tubes in a styrofoam container, which covered half of the tube surface). The first display that was produced immediately after the cold treatment was one of maximum duration, i.e. similar to the very first display of a still inexperienced individual. Apparently, exposure to the low temperature had knocked out the underlying neural system in control of the habituated display-duration, probably high-level inhibitions. But not irreversibly, the effects had vanished completely after a stay of 5-10 minutes at room temperature (Van den Assem et al. 1984).

**FEMALE RECEPTIVITY AND COPULATION**

Onset of receptivity in *Nasonia* females never occurs at random points in a display but always in synchrony with first headnods. This suggests that the additional nods in all series are not associated with pheromone discharge. Receptivity brings about a change in female posture: she raises her abdomen to expose the genital orifice. At the same time she lowers her antennae and draws them tightly in to the front of her head. It is this latter movement that promptly sends the male backward into the copulatory position. The movement’s signal function was verified with a dummy with fake antennae that could mimic the movements of real antennae, operated by remote control (Van den Assem & Jachmann 1982). The same dummy offered a possibility to study the temporal relationship of signal and reaction. Setting off the signal at other points than first nods did stop the male’s performance but the backing up response failed to appear completely or with certain latencies. In fact, a quantitative relationship was found between duration of latency and timing of the signal relative to the display. These results produce experimental evidence for periodic endogenous changes during successive courtship cycles (Jachmann & Van den Assem 1993).

A virgin *Nasonia* female is able to signal receptivity twice. First, when courted for the first time by a conspecific male. Once receptive, copulation will follow immediately. Secondly, during the bout of post-copulatory courtship produced by the same male after his immediate return to the frontal courtship position. There is no backing up with the second signal, the male dismounts at once. In experimental situations with longer intervals between first and second signal (the male was brushed off immediately following copulation), the second signal appeared after far more pheromone discharges than in normal procedures. However, the final effects were similar: signalling twice ‘switched off’ females for the time being (days or weeks); third signals were practically absent.

The switched-off condition seems quite likely an effect of copulation or insemination. The more so because the copulator gains most: sperm competition will not arise. Yet, it may have different roots. Once the female has signalled receptivity, brushing off can stop the male’s backing-up response. The same procedure can be repeated with a second male (and a third). In those cases neither insemination nor copulation occurred but nevertheless females proved to be ‘switched off’ and remained unreceptive over extensive periods. (The phenomenon is not restricted to *Nasonia*. Similar effects were found in other pteromalids). It is of interest that the switched-off period proved far shorter lived in females taken from our LeidenLabII culture at a later date. Over time (a period of 20 plus years) several processes had changed gradually (Van den Assem & Jachmann 1999).

Two further aspects of receptivity and copulation must be mentioned. With the onset of receptivity, the female becomes fully immobile and is apparently no longer reacting to external stimuli. If the male is brushed off before he can make genital contact, she will stay in the copulation posture for exactly the same time as the duration of a normal copulation. During this period the female can be shuffled around over the substrate. If this treatment stops before the expected end of the immobility stance, she will start moving about at the predicted moment; no shying reactions present. Shying reactions will appear if the shuffle treatment goes on for a second too long. Duration of copulation is species-characteristic; in *Nasonia* species *ca.* 12 s (22°C), in a related species, *Lariophagus distinguendus* in which the peculiar phenomenon was first noticed, it is far longer (60 s on average) and so are the freezing/shuffling periods.

It is the female that puts an end to copulation. Sometimes a male is late with establishing genital contact; *e.g.* because of an initially incorrect orientation. In such cases the female may soon
start walking away, dragging the male behind and thus severing the connection. In the case of
sneakers (that frequently take precedence of copulation on the male that induced receptivity by
proper courtship production) the later coming male is frequently too late to establish full or proper
contact; hence, for him incomplete insemination, or no insemination at all. Sneakers are kind of
sexual parasites, which prove that males are able to copulate without having courted first. They
take up the copulation position at the rear straight away but only if the courtship position is no
longer vacant (Fig. 3). As soon as the female adopts the copulation posture the sneaker makes
genital contact. A sneaker may soon after act as a courter and be sneaked itself. Sneaking is a
widespread phenomenon.

DESCRIPTION OF THE NASONIA COURTSHIP REPertoire

A male’s first reaction to the presence of a female is an increase in frequency of antennal
vibrations. Next, he turns and approaches. If the female moves away, he will follow in pursuit,
with vibrating wings. These vibrations are coupled to sounds that may serve as early warning:
females that are mounted by a mute male jump away or show death feigning upon being touched,
both reactions that preclude or delay the start of normal courtship procedures (Van den Assem &
Putters 1980).

A pursuing male may mount on the female from any direction. Usually he does so from the
rear, moves to the front and places the forefeet on the female’s head. This position is characteristic
for Nasonia and relatives. His arrival at the front immobilises the female. Consistent mobility (in a
small minority) delays the onset of courtship; reappearance of mobility later on will interrupt an
ongoing display.

The display starts with the antennae above and in front of the female’s head, (the angling
position); the flagellae in vibrating motion. Most following antennal movements are performed at a
high speed, some hardly perceptible with the naked eye. From the angling position, the antennae
are moved backward and upward until perpendicular to the body axis and more or less parallel to
the substratum. Next, they move downward, bringing scapes and flagellae in front of the female’s
head. This position is kept for a moment; it puts an end to what will be called phase-1. Phase-2
follows next, the antennae go again sideways and up, (it is difficult to determine how far, but at
least above the horizontal), and down again, all in one flash, and then return to the angling
position. The upward component accompanies the head’s drawing-in movement at the onset of the
first headnod (see next paragraph); the downward flick is in synchrony with the onset of the head-
raising component.

Head nods appear in series. The first, relatively slow nod starts at the onset of the phase-2
movements: the head seems to be ‘tucked-in’. The direction of movement is immediately reversed
with the phase-2 downward flick. The head is lifted relatively slowly, with the now extruded
mouthparts close to the female’s antennae, and remains stationary for a short moment at the
highest point, the ‘chin part’ elevated. The mouthparts are drawn in and the head moves back into
the initial position. These motor patterns are illustrated in Fig. 2 in Barrass (1960). The additional
nods in the same series proceed at a higher speed than the first one, and they lack the ‘tucking-in’
component. Mouthpart extrusions accompany every nod. A pause separates the series of nods from

Figure 3. A courting Nasonia couple and a ‘sneaky’ male
Figure 4. Sonogram of a courting Nasonia vitripennis male

the antennal sweep at the start of the next display cycle. The antennae are kept in the angling position during the pause. Successive cycles increase slowly in duration. Successive headnods series may vary in number of nods; on this point there are differences between the three species, see below.

Additional movements appear during a display, some during the pauses in between headnod series, some irregularly, some all the time. Wing vibrations are present throughout, visible as short buzzes. The position of the wings varies from lying flat on the male’s dorsum to fully extended, perpendicular to the body axis. Differences in wing postures do not affect sound production. Bouts of fore and/or hind leg movements may appear during pauses but do not seem to follow strict rules; they are difficult to quantify but may serve as discriminating characters (see below). Finally, there exist special head-nodding movements, which are not associated with mouthpart extrusion and do not appear in clusters (so-called ‘minus’ nods). They are conspicuously present in longicornis, far less so in giraulti, and absent in vitripennis. In particular at the end of a display these movements may appear as fully-fledged nods, similar to additional nods of headnod series, but lacking mouthpart extrusion. At the start of a display they are small-amplitude nods mostly.

**SPECIES-CHARACTERISTIC COMPONENTS OF NASONIA DISPLAYS**

*Nasonia vitripennis*

Males pursue females vigorously. Head nodding starts almost immediately because phase-1 antennal movements are lacking prior to the first series. In phase-1, the downward pointing antennae are kept rather wide apart in front of the female’s head. Typically, the second headnod series contains fewer nods (one or two less) than the first series. From the third series onward numbers tend to increase slowly. Sonagram tracings of courtship sounds show continuous repetition of a horseshoe like pattern, at about 3 per 2 s (at 22°C), with the start and end at ca. 50 Hz, and a peak frequency of about 400 Hz (Fig. 4). (No records are available of sound production of the two other species). The forefeet make rather inconspicuous stepping movements, usually during the pauses in between headnod series. Average duration of 1st and 2nd cycle is 7.5 and 7.5 s; average number of nods in 1st and 2nd cycle is 5-7 and 4-5.

*Nasonia giraulti*

Males pursue females not as vigorously as vitripennis do. Sometimes, mounting suggests an aggressive quality, reminiscent of the assault jump that occurs in encounters between males. Once in courtship position, the antennae are in the angling mode, with vibrating flagellae. The display starts with a full phase-1/phase-2 performance. In the downward pointing frontal stretch that follows the sideways movement, the antennae are parallel and close together, with scapes and flagellae in one line. The tips of the flagellae make contact (or nearly so) with the female’s mouthparts. (Males seem to search for a touch or near-touch before they proceed. On females that tuck in their head with ‘chin part’ to the thorax as a reaction to being mounted, males bend far over, reaching out for a contact, and thus delay the onset of head nodding). The stretch position is kept for a short while. From here on, the further movements are very hard to follow because of
high-speed performance. The antennae seem to be raised again, pointing laterally, and immediately brought down again, aside of the female antennae, and finally end up in the angling position. The 2nd phase of the antennal movements coincides with the onset of head nodding; sideways raising with the inward motion of the head, downward sweep with onset of head raising and mouthpart extrusion. Within series, the antennae move up and down; with every successive nod the scapi are raised gradually.

In general, the number of nods per series follows the *vitripennis* pattern but on a higher level. The additional nods in early series are often shallow and difficult to count. ‘Minus nods’ may appear solitary or in pairs, but never in long sequences as in *N. longicornis*. A single minus nod appears just ahead of the antennal downward stretch, pairs about midway in the pauses between headnod series.

Forefeet movements appear frequently just ahead of the antennal downward stretch; they give the impression of ‘hammering’ instead of ‘stepping’ (as in *vitripennis*). The differences are not impressive, if real at all. Hind-feet strokes along the leading edge of the female’s fore wings are a *giraulti* speciality. They appeared in all displays, either single or in short bouts, during the pauses in between headnod series, or ahead, or during the antennae-pointing-downward-in-contact-with-female-mouthparts phase. Average duration of 1st and 2nd cycle is 22.5 and 23 s; average number of nods in 1st and 2nd cycle is 12 and 11.

*Nasonia longicornis*

Upon arrival at the courtship position, the antennae are in the angling position. Meanwhile, the male starts nodding his head, almost imperceptibly at first, more distinct later on. Mouthpart extrusions remain absent. At the same time, the antennae gradually move sideways, until perpendicular to the length axis of the body and about parallel to the substrate. From this position they move downward until pointing obliquely to the front, with scapes and flagellae in a straight line. Antennae remain far more apart than in *giraulti*, and there is no contact with the female’s mouthparts or clypeus. After a short pause the antennae are raised again, up to horizontal, and flick low, in synchrony with the first headnod, which start without a separate ‘tucking in’ movement, probably because of the fluent transition from one nod to the other. Similar antennal movements precede all following headnod series. The minus nods become more and more distinct in the course of the display and in the end may attain the same amplitude as headnods with extrusion.

![Figure 5. Reciprocal crosses of Nasonia vitripennis and Nasonia longicornis used to generate hybrid offspring. F1 hybrid females were propagated as virgins to produce F2 hybrid sons only. V: N. vitripennis, L: N. longicornis. Letters in brackets identify type of cytoplasm.](image-url)
Series of headnods with mouthpart extrusion include only a few nods at the start of the display (1, 2 or 3); these numbers remain on the same level throughout, or increase slowly.

Forefeet rubs are a *longicornis* speciality. The front tarsi move sideways over the female’s eyes (and sometimes beyond, with a loss of contact) in series of exaggerated, rapid rubbing movements. Left and right foreleg alternate in the performance.

Average duration of 1st and 2nd cycle is 12 and 16 s, average number of nods in 1st and 2nd cycle is 1.6-2.3 and 2.0-2.6.

**Hybrid courtship**

Cured lines of *N. vitripennis* and *N. longicornis* were used for making bidirectional interspecific crosses. Hybrids were classified as VL or LV, with reference to resp. the paternal and maternal line (Fig. 5). The F1 hybrid daughters were propagated as virgins; the displays of F2 hybrid sons were analysed (Beukeboom & Van den Assem 2001). Hybrid displays were expected to turn out as intermediate between the parental species, and mutually more or less similar since VL and LV sons receive about equal V and L genomic contributions through their hybrid mothers. For several components the intermediate character of hybrid displays was confirmed but in addition there was a significant bias towards the paternal species, the so-called ‘grandfather effect’ (due to haplodiploid sex determination, *Nasonia* males are fatherless but do have a grandfather). Hybrid displays were not intermediate for number of cycles until dismounting. Usually, far more cycles than in pure-line displays were involved, not unlikely the effect of a malfunctioning endogenous control. Also various qualitative aspects point to malfunctioning: on average, males took longer before they mounted on a female – if at all; many did not follow in pursuit, or turned away prematurely. Once mounted, they frequently failed to orient properly. Some males did mount correctly but failed to stop at the front and walked off straight away, a procedure never observed in pure-line males.

A ready explanation of the grandfather effect was not on hand but some sort of nucleo-cytoplasmic interaction (Breeuwer & Werren 1993) seemed to be a likely cause. In interspecific hybrids, the paternally donated genes must operate in an heterospecific environment, the maternal, heterospecific cytoplasm; the effects on subsequent courtship behaviour are unpredictable. The hypothesis was put to a test by hybridising introgressed lines. In the hybrid sons the paternally donated nuclear genes and the maternal cytoplasm are conspecific. A schematic clarification, the type of cytoplasm is in square brackets; pure lines V[V], L[L]; interspecific hybrids VL[L], LV[V]; introgressed lines V[L], L[V]; introgressed hybrids VL[V], LV[L]. If the grandfather effect were the outcome of heterospecific interactions of the kind outlined above, it should be absent in VL[V] and LV[L] males. However, it was not (Beukeboom & Van den Assem 2002). The search is still on.

**REFERENCES**


