Allohormones and sensory traps: a fundamental difference between hermaphrodites and gonochorists?

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Summary

Multiple mating, sperm storage and sperm digestion cause uncertainty about the fate of donated sperm. In invertebrates these are common processes and increase the selective pressure for tactics that enhance fertilization success. Hence, to assure that donated sperm will be used for their rightful purpose, many different strategies can evolve. For example, biochemical substances — such as pheromones and allohormones — can be employed to improve the chances of fertilizing the partner's eggs. Several recent examples of such substances in gonochoric and hermaphroditic invertebrates are reviewed here. Moreover, I argue that a simple but fundamental difference may exist in the evolution of allohormones between the two modes of gender expression, primarily based on the differences in gene expression between species with separate sexes and hermaphrodites. This idea suggests that a biochemical sensory trap could occur more readily in hermaphrodites, and, depending on the mode of transfer of these allohormones, this should be reflected in a different speed of allohormone evolution.

Key words: Evolution, gender expression, pheromone, sex peptide, sexual selection

Biochemicals Influencing Fertilization

Sexual selection favours tactics that increase an individual's fertilization success and offspring production, both in gonochorists (species with separate sexes) (e.g., Andersson, 1994) and simultaneous hermaphrodites (e.g., Michiels, 1998). Typically, sperm donors compete for fertilization opportunities to assure their paternity (e.g., Birkhead and Møller, 1998), although some examples of the reverse situation exist (e.g., Gwynne and Simmons, 1990; Arnqvist et al., 2003). The selection for paternity assurance is especially strong when sperm competition is fierce due to multiple mating, sperm digestion or ejection, and sperm storage (e.g., Parker, 1970). As a result of the attempts to increase fertilization chances of the donated sperm, energetically costly ejaculates and mating behaviours evolve (e.g., Dewsbury, 1982). Moreover, when these tactics negatively affect the sperm recipient, a sexual conflict may ensue (e.g., Chapman et al., 2003).

There are many ways in which animals can increase their fertilization success. Overt behaviours include mate guarding, physical male–male competition, and frequent intra-pair copulations. In addition, more concealed processes take place. In many of these covert paternity assurance strategies, biochemical substances

are emitted or transferred. These biochemicals can take the form of pheromones, which are detected by specialized organs (e.g., Karlson and Lüscher, 1959). Such substances can also bypass sensory organs and act directly on the physiology or behaviour of the recipient, in which case they are referred to as allohormones, to distinguish them from pheromones (see Koene and Ter Maat, 2001, 2002).

Both pheromones and allohormones can play key roles in the outcome of sperm competition and mate choice, and can thus have a large impact on fertility and reproductive success. As illustrated below with some recently discovered examples, these biochemical substances can occur both in gonochoric and hermaphroditic species and serve similar functions. The evolutionary forces that shape pheromones and allohormones also seem similar. For pheromones this may be true, although theoretically their evolution could be limited in simultaneous hermaphrodites if animals are attracted by their own pheromones. Likewise, simultaneous hermaphrodites need to be extra careful with the transfer of allohormones to their partner to avoid exposing themselves to their own manipulative products (which requires strict compartmentalization). A more fundamental difference might exist between the evolution of allohormones in hermaphrodites and gonochorists. As argued below, this difference potentially stems from the genetics underlying the expression of a hermaphroditic or gonochoric gender, and predicts that allohormones more often cause "sensory traps" — by exploiting pre-existing sensory biases — in hermaphrodites.

In gonochoric species key genes that specifically regulate one sexual function are active, while the genes for the other sex are either silenced (e.g., Arthur et al., 1998; Werren and Beukeboom, 1998) or absent, e.g., genes on sex chromosomes in the homogametic sex. This also holds for cases where a species consists of hermaphroditic individuals and individuals with one sex, as occurs in *Caenorhabditis elegans* (Hodgkin, 1999; Raymond et al., 1998). In several invertebrates the differential expression of sex genes has been shown to be primarily determined by the ratio of sex chromosomes and autosomes (e.g., Werren and Beukeboom, 1998; Hodgkin, 1999; Reinke et al., 2004), while in vertebrates the expression of the SRY gene on the Y-chromosome is the primary sex determinant (e.g., Koopman et al., 1991). Thus, because female genes are silenced in males, in order to manipulate females, males need to either "invent" manipulative substances or re-activate silenced genes that code for products involved in the regulation of female processes.

On the contrary, in simultaneous hermaphrodites the male and female functions are regulated simultaneously. Therefore, male and female genes are expressed (in different compartments) of each individual (Michiels, 2000). As a consequence, hormones and neuropeptides, as well as their receptors, specifically involved in male or female processes are produced in every individual. The combination of the presence of the receptors in the partner and the ability to produce the substances activating such receptors allows for "easy access" to manipulation. In other words, substances that are normally used to regulate female reproduction in the producer can potentially be exploited as manipulative substances that are transferred during mating much more easily in hermaphrodites than in species with separate sexes. Such sensory exploitation can then result in a sensory trap because the manipulative substance is also essential for normal regulation of the female reproductive system (e.g., Eberhard, 1996).

With the above-mentioned idea in mind, some of the recently discovered biochemical substances involved in reproduction are briefly reviewed. For these proceedings, the work is limited to that done on invertebrates and especially done by participants of the 10th International Congress on Invertebrate Reproduction and Development that took place in Newcastle-upon-Tyne (18–23 July 2004). The aim in this review is therefore twofold. Firstly, to highlight some recent examples of ways in which pheromones and allohormones can act, and thereby illustrate the distinction between the two classes of bioactive substances; secondly, using these examples, to evaluate the validity of the proposed difference in evolution of allohormones in gonochoric and hermaphroditic species and suggest some ways to test the predictions following from this theoretical difference.

Pheromones: Stimulation, Attraction and Repulsion

Pheromones are substances that are released to the outside of one animal and are detected by specialized sensory organs in another member of the same species where they induce a specific reaction (Karlson and Lüscher, 1959). They are often released into the animal's environment, in which case they are either air- or water-borne. When they need to be brought into direct contact with the recipient, they are referred to as contact pheromones (e.g., Koene and Ter Maat, 2001). Although it should be noted that pheromones are not limited to reproductive processes, they are probably most notorious for playing an essential role in

reproduction. Here, the focus is on only a few recently discovered pheromones involved in reproductive processes.

In marine invertebrates, copulation and spawning are often linked to tidal and/or lunar cycles (e.g., Bentley et al., 2001; Hodgson, 1999). This assures that gametes are mixed at the moment when conditions are optimal for gamete survival, fertilization and/or larval survival. Synchronized mass spawning can be the result, thus enhancing fertilization chances of the eggs and increasing sperm competition (e.g., Ball and Parker, 1996). Such spawning *en masse* can be enhanced by pheromones that induce gamete release. This happens, for instance, in corals (e.g., *Euphyllia ancora*: Twan et al., 2003) and polychaete worms (e.g., *Arenicola marina*: Hardege and Bentley, 1997; *Nereis succinea*: Ram et al., 1999). The combination of pheromones and tidal/lunar effects may also explain the occurrence of synchronous spawning of multiple species of corals (e.g., Guest et al., 2002).

Of course, before spawning or copulating, many free-living organisms first need to find a mating partner. Again this is often mediated via pheromones, and there are countless examples. Recently, it has been shown that females of the crayfish *Pacifastacus leniusculus* (Stebbing et al., 2003) and the scale worm *Harmothoe imbricata* (Watson et al., 2000) use pheromones to attract males. Likewise, hermaphroditic sea slugs of the genus *Aplysia* use a pheromone that is released from their egg cordon which attracts potential mates and stimulates them to lay eggs on the same site (Painter et al., 2004). But besides attracting mating partners, a pheromone can also be used for deterring. A nice example of this is found in the desert locust, *Schistocerca gregaria*. Males of this species emit a pheromone that deters other males during mating. Thus, this courtship-inhibition pheromone keeps male competitors away from the female and thereby reduces sperm competition (Seidelmann and Ferenz, 2002). Because this remating inhibition may limit (cryptic) female choice, this chemically enhanced mate guarding may result in a sexual conflict.

Allohormones: Stimulation, Induction and Inhibition

Allohormones are defined as substances that are transferred from one individual to another member of the same species and induce a direct physiological effect in the recipient, bypassing sensory organs (Koene and Ter Maat, 2001, 2002; Koene, 2004). There are different ways to transfer allohormones and they can have different effects on the recipient.

Although allohormones are also not limited to reproductive processes, several of their modes of action and transfer are addressed here by focusing on reproductive processes.

Transfer of allohormones via the semen is common and can have a range of effects, including reduction of receptivity, induction of egg laying, stimulation of sperm storage, inhibition of sperm digestion, and immunosuppression (e.g., Koene and Ter Maat, 2001; Koene, 2004). When these effects negatively affect the female, a sexual conflict arises (e.g., Chapman et al., 2003). In the cuttlefish *Sepia officinalis* egg laying is induced by copulation, an effect which is believed to be caused by a seminal product, although this remains to be experimentally demonstrated (Bernay et al., 2004). Seminal products, such as sex peptides and ductus ejaculatorius peptide, have been studied in great detail in the fruit fly *Drosophila melanogaster* (for a recent review see Kubli, 2003). They have been shown to have a variety of effects, including an increase in egg laying and a reduction in remating (Fan et al., 2000).

Males can also use allohormones to inhibit pheromone production in the female, thus making her less attractive for competing males. Mating has been shown to terminate pheromone production in females of several moth species. One of these species is *Helicoverpa armigera* in which it has been demonstrated that the pheromone-suppression peptide *Hez*PSP is transmitted via the semen. This allohormone inhibits pheromone production by interfering with the pheromone synthesis activating neuropeptides (Fan et al., 2000; Eliyahu et al., 2003). Another unusual substance, transferred by the male tick upon mating, is the so-called engorgement factor (*Ah*EF), which induces a rapid feeding phase in the female (*Amblyomma hebraeum*: Weiss and Kaufman, 2004). This engorgement process is responsible for the enormous increase in female weight — 10 to 14 times the unfed weight — and is essential for the production of eggs.

In hermaphroditic animals allohormones are also used to influence the fate of the donated sperm. For example, the pond snail *Lymnaea stagnalis* seems to influence the partner via seminal products. The receipt of semen has been shown to initiate egg laying in virgin animals (Koene and Ter Maat, 2004; Van Duivenboden, 1983), but also seems to feminize the mating partner later in life (Koene et al., submitted). These increases in the female function have been shown to occur at the expense of growth and prostate gland development of the sperm recipient (Koene and Ter Maat, 2004; Koene et al., submitted). The importance of these products for fertilization success may be

the ultimate reason why these snails only mate in the male role when enough seminal fluid is available in the prostate gland (Koene and Ter Maat, 2005). The findings also hint at the existence of a conflict over sex allocation between the mating partners, although it remains to be demonstrated that this is caused by an allohormone.

Allohormones can also be transferred hypodermically, as happens in earthworms and land snails. Common earthworms (*Lumbricus terrestris*) pierce their partners with 40 to 44 copulatory setae to inject a substance into the skin (Koene et al., 2002). Experimental removal of the copulatory setae revealed that these setae — or the substance that they inject — assure that more sperm is stored and that sperm is distributed equally in all four spermathecae of the partner (Koene et al., 2005). This finding indicates that earthworms may induce their partners to alter sperm storage in order to increase the fertilization chances of the donated sperm.

Individuals of the common garden snail *Cantareus aspersus* (previously *Helix aspersa*) use the so-called love dart to increase the chances of their donated sperm. This dart is stabbed into the mating partner before spermatophore transfer and carries an allohormone that inhibits sperm digestion (Koene and Chase, 1998a, 1998b). Increased sperm storage and paternity are the result (Rogers and Chase, 2001, 2002; Landolfa et al., 2001). Many other species of land snails forcefully stab one or more love darts into their mating partners (e.g., Koene and Chiba, unpublished; Koene, 2005; Koene and Muratov, 2004; Reyes Tur et al., 2000; Baminger et al., 2000). When these dart-possessing snails were compared in an inter-species study, co-evolution between darts and spermatophore-receiving organs was revealed (Koene and Schulenburg, 2005). To the best of my knowledge, this is the first study that supports the effect that sexual conflict can have on the evolution of the reproductive morphology in simultaneous hermaphrodites (for gonochorists, see Arnqvist and Rowe, 2002).

One exciting and novel method of allohormone transfer that has not received sufficient attention is an idea proposed by Buckland-Nicks (1998). He observed that the large, non-fertilizing spermatozoa (parasperm) of the prosobranch *Fusitriton* release substances into the seminal fluid. Rather than only transferring seminal fluid produced by a prostate gland upon sperm donation, these parasperm seem able to continue releasing substances into the female reproductive tract after transfer. Of course, it remains to be demonstrated that the released substances affect fertilization success. However, encouraging evidence comes from another

prosobranch, *Viviparus ater*. This species has been shown to increase the transfer of parasperm (oligopyrene sperm) in response to higher sperm competition (Oppliger et al., 1998). Additionally, the notion that these large non-fertilizing sperm are not nuptial gifts is supported by the fact that hermaphroditic species also produce them (e.g., Jespersen et al., 2002). Although the exchange of nuptial gifts between hermaphroditic mating partners potentially represents a form of sperm trading (e.g., Michiels, 1998), it ultimately results in a net gain of zero or less (Koene and Chase, 1998a; Koene et al., submitted).

Hermaphrodites vs. Gonochorists

With some recently discovered examples, I illustrated above how pheromones and allohormones are used by gonochorists as well as simultaneous hermaphrodites. Both types of gender expression use pheromones to stimulate, attract or repulse mating partners, while allohormones seem to be used in a more initiating or manipulating way. In the introduction I suggested that for allohormones there seems to exist a simple but fundamental difference between simultaneous hermaphrodites and gonochorists. This difference seems due to the underlying genetics because in simultaneous hermaphrodites the genes for both male and female regulatory substances are active within each individual, while the genes for one sex are suppressed or even missing (in the homogametic sex) in gonochorists (see also Michiels, 2000). Hence, simultaneous hermaphrodites seem to have relatively easy access to the substances that are used in the regulation of the (partner's) female reproductive system. The current evidence based on gene expression seems to lend support to this idea, but what are the implications and predictions that arise and how can they be tested?

If hermaphrodites indeed use allohormonal substances that are directly derived from female hormones or neuropeptides, this may represent an inescapable manipulation (i.e., sensory trap). Such biochemical sensory exploitation would occur when the regulation of the female reproductive system depends on the same substance that is used as a stimulus by the sperm donor (see also Eberhard, 1996). Interestingly, sensory exploitation in species with separate sexes seems usually based on stimuli from a completely different context, which have no direct relation to reproduction (reviewed by Christy, 1995). For example, such stimuli can be visual (crabs, fire flies: e.g., Christie, 1995), auditory (frogs: e.g., Ryan, in press), olfactory (butterflies: e.g., Christie, 1995), mechanical (damselflies:

Córdoba-Aguilar, 2002), or food (crickets: Sakaluk 2000). All these, often exaggerated, stimuli make use of pre-existing sensitivities that evoke responses which indirectly increase male fertilization success. Evidently, all these mechanisms can also work in simultaneous hermaphrodites (although not many studies have investigated this). But in addition, hermaphrodites seem to have "easy access" to exploiting the pre-existing sensitivity of their partners to the substances used to regulate reproductive processes.

In the above case, reducing sensitivity to the allohormone could decrease the manipulative effect but would at the same time reduce the efficiency of the female reproductive system. Hence, such a sensory trap would actually limit the potential for counter-adaptive co-evolution at the biochemical level. As a consequence, counter-adaptations may instead be mainly achieved at morphological and behavioural levels. What emerges as a general prediction is that manipulative allohormones that are produced by hermaphrodites should be more similar to substances used in the regulation of female reproduction than in gonochorists (where such substances are more likely to arise by chance, although gene re-activation is also possible). For testing this prediction allohormones from gonochoric and hermaphroditic species need to be compared systematically. Ideally, this comparison would be done in closely related species within a genus, but alternatively less related species can be compared provided that phylogenetic distance between the species is taken into account.

Finally, reproductive biochemicals (including allohormones) have been shown to evolve rapidly in species with separate sexes, which can be explained by the continuous adaptation and counter-adaptation between male manipulative substances and female receptors (e.g., Begun et al., 2000; Swanson and Vacquier, 2002; Haygood, 2004). In this context, an interesting question to address in the future is how fast such substances evolve in simultaneous hermaphrodites. Based on the above, one would predict that allohormones present in the seminal fluid may evolve slower in hermaphrodites that in separate sex species, because in the former the possibilities for counter-adaptations at the biochemical level are very limited. But, of course, for different modes of transfer of allohormones — such as hypodermic injection — counter-adaptations are probably much less constrained and might therefore display a very high rate of evolution.

Acknowledgements

References

Andersson, M., Sexual Selection, Princeton University Press, 1994.

Arnqvist, G. and Rowe, L., Antagonistic coevolution between the sexes in a group of insects. Nature, 415 (2002) 787–789.

Arnqvist, G., Jones, T.M. and Elgar, M.A., Insect behaviour: Reversal of sex roles in nuptial feeding. Nature, 424 (2003) 387.

Arthur, B.I., Jallon, J.M., Caflisch, B., Choffat, Y. and Nothiger, R., Sexual behaviour in *Drosophila* is irreversibly programmed during a critical period. Curr. Biol., 8 (1998) 1187–1190.

Ball, M.A. and Parker, G.A., Sperm competition games: External fertilization and '"adaptive"' infertility. J. Theor. Biol., 180 (1996) 141–150.

Baminger, H, Locher, R. and Baur, B., Incidence of dart shooting, sperm delivery, and sperm storage in natural populations of the simultaneously hermaphroditic land snail *Arianta arbustorum*. Can. J. Zool., 78 (2000) 1767–1774.

Begun, D.J., Whitley, P., Todd, B.L., Waldrip-Dail, H.M. and Clark, A.G., Molecular population genetics of male accessory gland proteins in *Drosophila*. Genetics, 156 (2000) 1879–1888.

Bentley, M.G., Olive, P.J.W. and Last, K., Sexual satellites, moonlight and the nuptial dances of worms: The influence of the Moon on the reproduction of marine animals, Earth Moon Planets, 85 (2001) 67–84.

Bernay, B., Gagnon, J. and Henry, J., Egg capsule secretion in invertebrates: a new ovarian regulatory peptide identified by mass spectrometry comparative screening in *Sepia officinalis*, Biochem. Biophys. Res. Commun., 314 (2004) 215–222.

Birkhead, T.R. and Møller, A.P., Sperm Competition and Sexual Selection, Academic Press, 1998.

Buckland-Nicks, J., Prosobranch parasperm: Sterile germ cells that promote paternity? Micron, 29 (1998) 267–280.

Chapman, T., Arnqvist, G., Bangham, J. and Rowe, L., Sexual conflict. Trends Ecol. Evol., 18 (2003) 41–47.

Christy, J.H., Mimicry, mate choice, and the sensory trap hypothesis. Am. Nat., 146 (1995) 171–181.

Córdoba-Aguilar, A., Sensory trap as the mechanism of sexual selection in a damselfly genitalic trait (Insecta: Calopterygidae). Am. Nat., 160 (2002) 594–601.

Dewsbury, D.A., Ejaculate cost and male choice. Am. Nat., 119 (1982) 601–610.

Eberhard, W.G., Female control: sexual selection by cryptic female choice, Princeton University Press, New Jersey, 1996.

Eliyahu, D., Nagalakshmi, V., Applebaum, S.W., Kubli, E., Choffat, Y. and Rafaeli, A., Inhibition of pheromone

biosynthesis in *Helicoverpa armigera* by pheromonostatic peptides. J. Insect Physiol., 49 (2003) 569–574.

Fan, Y.L., Rafaeli, A., Moshitzky, P., Kubli, E., Choffat, Y. and Applebaum, S.W., Common functional elements of *Drosophila melanogaster* seminal peptides involved in reproduction of *Drosophila melanogaster* and *Helicoverpa armigera* females. Insect Biochem. Mol. Biol., 30 (2000) 805–812.

Guest, J.R., Chou, L.M., Baird, A.H. and Goh, B.P.L., Multispecific, synchronous coral spawning in Singapore. Coral Reefs, 21 (2002) 422–423.

Gwynne, D.T. and Simmons, L.W., Experimental reversal of courtship roles in an insect. Nature, 346 (1990) 172–174.

Hardege, J.D. and Bentley, M.G., Spawning synchrony in *Arenicola marina*: evidence for sex pheromonal control. Proc. R. Soc. Lond. B, 264 (1997) 1041–1047.

Haygood, R., Sexual conflict and protein polymorphism, Evolution, 58 (2004) 1414–1423.

Hodgkin, J., Sex, cell death, and the genome of *C. elegans*. Cell, 98 (1999) 277–280.

Hodgson, A.N., The biology of siphonariid limpets (Gastropoda : Pulmonata). Ocean. Mar. Biol., 37 (1999) 245–314.

Jespersen, A., Lützen, J. and Morton B., Ultrastructure of dimorphic sperm and seminal receptacle in the hermaphrodites *Barrimysia siphonosomae* and *Pseudopythina ochetostomae* (Bivalvia, Galeommatoidea). Zoomorph., 121 (2002) 159–172.

Karlson, P. and Lüscher, M., "Pheromones": a new term for a class of biologically active substances. Nature, 183 (1959) 55–56.

Koene, J.M., Immunomodulation, allohormones and fertility. Med. Hyp., 63 (2004) 1084–1085.

Koene, J.M., Love darts in land snails. Invert. Biol., 124 (2005) 1.

Koene, J.M. and Chase, R., The love dart of *Helix aspersa* Müller is not a gift of calcium. J. Moll. Stud., 64 (1998a) 75–80.

Koene, J.M. and Chase, R., Changes in the reproductive system of the snail *Helix aspersa* caused by mucus from the love dart. J. Exp. Biol., 201 (1998b) 2313–2319.

Koene, J.M. and Muratov, I.V., Revision of the reproductive morphology of three *Leptaxis* species (Gastropoda, Pulmonata, Hygromiidae) and its implication on dart evolution. Malacologia, 46 (2004) 73–78.

Koene, J.M., Pförtner, T. and Michiels, N.K., Piercing the partner's skin influences sperm storage in earthworms. Behav. Ecol. Sociobiol., in press.

Koene, J.M. and Schulenburg, H., Shooting darts: coevolution and counter-adaptation in hermaphroditic snails. BMC Evol. Biol., 5 (2005) 25.

Koene J.M., Sundermann, G. and Michiels, N.K., On the function of body piercing during copulation in earthworms, Invert. Reprod. Develop., 41 (2002) 35–40.

Koene, J.M. and Ter Maat, A., "Allohormones": A class of bioactive substances favoured by sexual selection. J. Comp. Physiol. A, 187 (2001) 323–326.

Koene, J.M. and Ter Maat, A., The distinction between pheromones and allohormones. J. Comp. Physiol. A, 188 (2002) 163–164.

Koene, J.M. and Ter Maat, A., Sex role alternation in the simultaneously hermaphroditic pond snail *Lymnaea stagnalis* is determined by the availability of seminal fluid. Anim. Behav., 69 (2005) 845–850.

Koene J.M. and Ter Maat, A., Energy budgets in the simultaneously hermaphroditic pond snail, *Lymnaea stagnalis*: A trade-off between growth and reproduction during development. Belg. J. Zool., 134 (2004) 41–45.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. and Lovell-Badge, R., Male development of chromosomally female mice transgenic for Sry. Nature, 351 (1991) 117–121.

Kubli, E., Sex-peptides: seminal peptides of the *Drosophila* male. Cell. Mol. Life Sci., 60 (2003) 1689–1704.

Landolfa, M.A., Green, D.M. and Chase, R., Dart shooting influences paternal reproductive success in the snail *Helix aspersa* (Pulmonata, Stylommatophora). Behav. Ecol., 12 (2001) 773–777.

Michiels, N.K., Mating conflicts and sperm competition in simultaneous hermaphrodites. In Sperm Competition and Sexual Selection, T.R. Birkhead and A.P. Møller (eds.), Academic Press, 1998, pp. 219–254.

Michiels, N.K., Hermaphrodites: the long-term costs of being a short-term opportunist. J. Reprod. Devel., 46 (2000) 15–16.

Opplinger, A., Hosken, D.J. and Ribi, G., Snail sperm production characteristics vary with sperm competition risk. Proc. R. Soc. Lond. B, 265 (1998) 1527–1534.

Painter, S.D., Cummins, S.F., Nichols, A.E., Akalal, D.B.G., Schein, C.H., Braun, W., Smith, J.S., Susswein, A.J., Levy, M., De Boer, P.A.C.M., Ter Maat, A., Miller, M.W., Scanlan, C., Milberg, R.M., Sweedler, J.V. and Nagle, G.T., Structural and functional analysis of *Aplysia* attractins, a family of water-borne protein pheromones with interspecific attractiveness. Proc. Natl. Acad. Sci. USA, 101 (2004) 6929–6933.

Parker, G.A., Sperm competition and its evolutionary consequences in the insects. Biol. Rev., 45 (1970) 525–567.

Ram, J.L., Muller, C.T., Beckmann, M., and Hardege, J.D., The pheromone cysteine-glutathione disulfide ("Nereithione") activates nuptial behavior, spawning, and electrophysiological activity of *Nereis succinea* males. FASEB, 13 (1999) 945–952.

Raymond, C.S., Shamu, C.E., Shen, M.M., Seifert, K.J., Hirsch, B., Hodgkin, J. and Zarkower, D., Evidence for evolutionary conservation of sex-determining genes. Nature, 391 (1998) 691–695.

Reinke, V., Gil, I.S., Ward, S. and Kazmer, K., Genome-wide germline-enriched and sex-biased expression profiles in *Caenorhabditis elegans*. Development, 131 (2004) 311–323.

Reyes Tur, B., Velázques, A.F. and Cabrera, Y.O., Conducta de apareamiento y aspectos de la relación des sistema reproductor en *Polymita muscarum* Lea 1834 (Gastropoda: Pulmonata). Revista Biologí'a, 14 (2000) 160–166.

Rogers, D. and Chase, R., Dart receipt promotes sperm storage in the garden snail *Helix aspersa*. Behav. Ecol. Sociobiol., 50 (2001) 122–127.

Rogers, D. and Chase, R., Determinants of paternity in the

garden snail *Helix aspersa*, Behav. Ecol. Sociobiol., 52 (2002) 289–295.

Ryan, M.J., The evolution of behavior, and integrating it towards a complete and correct understanding of behavioral biology. Anim. Biol., in press.

Sakaluk, S.K., Sensory exploitation as an evolutionary origin to nuptial food gifts in insects. Proc. R. Soc. Lond. B., 267 (2000) 339–344.

Seidelmann, K. and Ferenz, H.M., Courtship inhibition pheromone in desert locusts, *Schistocerca gregaria*. J. Insect Physiol., 48 (2002) 991–996.

Stebbing, P.D., Bentley, M.G. and Watson, G.J., Mating behaviour and evidence for a female released courtship pheromone in the signal crayfish *Pacifastacus leniusculus*. J. Chem. Ecol., 29 (2003) 465–475.

Swanson, W.J. and Vacquier, V.D., Reproductive protein evolution. Ann. Rev. Ecol. Syst., 33 (2002) 161–179.

Twan, W.H., Hwang, J.S. and Chang, C.F., Sex steroids in scleractinian coral, *Euphyllia ancora*: Implication in mass spawning. Biol. Reprod., 68 (2003) 2255–2260.

Van Duivenboden, Y.A., Transfer of semen accelerates the onset of egg-laying in female copulants of the hermaphrodite freshwater snail, *Lymnaea stagnalis*. Int. J. Invert. Reprod., 6 (1983) 249–257.

Watson, G.J., Langford, F.M., Gaudron, S.M. and Bentley, M.G., Factors influencing spawning and pairing in the scale worm *Harmothoe imbricata (*Annelida: Polychaeta). Biol. Bull., 199 (2000) 50–58.

Weiss, B.L. and Kaufman, W.R., Two feeding-induced proteins from the male gonad trigger engorgement of the female tick *Amblyomma hebraeum*. Proc. Natl. Acad. Sci. USA, 101 (2004) 5874–5879.

Werren, J.H. and Beukeboom, L.W., Sex determination, sex ratios, and genetic conflict. Ann. Rev. Ecol. Syst., 29 (1998) 233–261.