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Does the scent of a potential mate prevent the resorption of oocytes by apoptosis in *Nauphoeta cinerea*?

Emma L. B. Barrett, Allen J. Moore and Patricia J. Moore

Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall, UK

Abstract We expect organisms to have evolved mechanisms to gather and use the information available within their environments, to steer resource investment decisions toward the traits that will give the greatest fitness returns. Pheromones are social signals, a common purpose of which is to act as indicators of mate presence. Consequently, pheromones have the potential to act as signals to increase or maintain reproductive investment over that of competing life-history traits. In the cockroach, *Nauphoeta cinerea* (Oliver), females pay costs of maintaining investment in reproduction when there are no males, and males produce pheromones that are known to effect female reproductive outcomes. Whether the pheromones have an influence on resource physiology is unknown. We subjected newly eclosed females to either a synthetic blend of male pheromones or control. We dissected females at 7, 12, 17 or 22 days. We measured apoptosis levels and size of all oocytes within the vitellerium, and measured dry fat body mass. Synthetic male pheromone blend did not have an effect on any measure of female reproductive or somatic resource physiology. Although negative results are always problematic, the success of the synthetic pheromone method in the past suggests that females may be insensitive to male pheromones in the pre-oviposition period, and may rely on mating stimulus rather than pheromone as the cue for oocyte maintenance and growth. Previous studies reporting effects of male pheromones on female reproduction suggest that the period of pheromone sensitivity may be between mating and birth.

Key words apoptosis, environment, oocyte, ovary, pheromone, predictable, trade-off

Introduction

The trade-off between reproduction and survival is a central tenet of life-history theory (Roff, 1992; Stearns, 1992). Trade-offs occur when the investment in one life-history trait is balanced by the divestment in another (trait is defined as a characteristic that is positively correlated with fitness when all other variables remain constant, Schluter *et al.*, 1991). Resources invested in reproduction, such as in eggs, are unavailable for other processes, such as immune function, and *vice versa*. Investing resources inappropriately can reduce fitness. Individuals that can respond to their environment with investment in the appropriate life-history traits should have the greatest fitness gains (Hill & Low, 1992; Ricklefs & Wikelski, 2002). Hence, we expect mechanisms to have evolved for animals to be able to accurately obtain information from their environments and use it to shape their life histories (e.g., Vasi *et al.*, 1994; Ylönen & Ronkainen, 1994).

Social environments are important to many animals as they can convey information about reproductive opportunity, and hence whether resources should be preferentially invested in reproduction at that time. In species that are not visually conspicuous, chemical signals

Correspondence: Emma L. B. Barrett and Patricia J. Moore, Centre for Ecology and Conservation, School of Biosciences, University of Exeter, Cornwall Campus, Penryn, Cornwall, TR10 9EZ, UK. Tel.: +44 1326 371878; fax: +44 1326 253638; email: elbb201@ex.ac.uk, P.J.Moore@exeter.ac.uk

are integral to social interactions (Ritchie, 2000; Wyatt, 2003). Pheromones are among some of the most reliable biological cues in the environment (Marcillac & Ferveur, 2007), and may even have hormone-like capabilities (Moore *et al.*, 2003), where the pheromones produced by one animal can directly affect the physiology of another (Apfeld & Kenyon, 1999; Assad *et al.*, 1997; Mahamat *et al.*, 1993; Moore & Moore, 2003; Stern & McClintock, 1998; Tanaka *et al.*, 2006; Tawfik *et al.*, 2000; Wedekind *et al.*, 1995). In species where the distributions of populations are patchy in time and space, pheromones may play an important cooperative role in the synchronization of reproductive investment with the presence of suitable reproductive partners (Assad *et al.*, 1997). Reproductive synchronization could allow animals to invest in reproduction only when it is required, and thus minimize the magnitude of trade-offs between life-history traits.

In the ovoviviparous cockroach, *Nauphoeta cinerea* (Oliver), there is circumstantial evidence to suggest that females may have evolved a mechanism to synchronize reproduction with the availability of males. There are three main requirements allowing such a mechanism to evolve; the existence of a male-derived signal, genetic variation in female reproductive traits, and selection upon them (Lynch & Walsh, 1998). There is evidence of all three in *N. cinerea*. First, unlike most other cockroach species, in *N. cinerea* males produce the sex pheromones (Moore *et al.*, 1995; Sreng, 1990, 2003). Second, it has previously been determined that genetic variation exists for reproductive physiology (Moore *et al.*, 2007). Third, there are well-documented costs of reproductive investment in the absence of mates within this species (Barrett *et al.*, *in press*; Moore & Moore 2001). When females mate after a period of enforced virginity they can experience a decrease in fecundity (Moore & Moore, 2001), alongside a decline in female longevity (Barrett *et al.*, 2009). There is considerable variation to the extents to which females pay these costs, and part of this variation can be explained by genetic differences (Moore *et al.*, 2007).

In the addition, there is also experimental evidence to suggest that female *N. cinerea* are sexually 'primed' by the male pheromones (Moore & Moore, 2003). Female *N. cinerea*, like many insects (Chapman, 1998), have the ability to reproduce parthenogenically (Corley & Moore, 1999). However, few actually do, as parthenogenesis is much less fruitful than sexual reproduction (Moore & Moore, 2003). Not all females within the population seem capable of asexual reproduction (Corley *et al.*, 1999; Corley & Moore, 1999), and within the females that are, clutch sizes are dramatically reduced compared with sexual conspecifics due to reduced embryonic viability (Corley *et al.*, 1999). Part of the reason that asexuality is a less successful means of reproduction appears to be that females require male pheromones to reproduce successfully (Moore & Moore, 2003). When females are prevented from mating in the presence of male odors they are more likely to start reproducing asexually, and have more asexual offspring than females reared without exposure to male odor (Moore & Moore, 2003). This suggests that females have evolved to be receptive to their social environments due to the life-history benefits of informed investment. The optimization of sexual reproduction appears to be at the cost of becoming partially dependent on male social signals for successful reproduction (Corley *et al.*, 2001; Moore & Moore, 2003).

We explicitly investigated whether female *N. cinerea* have evolved a mechanism to synchronize investment in oocytes with the availability of males. Unfertilized oocytes degrade in quality with time (Andux & Ellis, 2008; Fissore *et al.*, 2002; Moore & Sharma, 2005). In the absence of males, females may resorb resources from oocytes via apoptosis, or maintain oocytes at the risk that oocytes may pass a developmental point of no return past which resorption may no longer be an option and oocytes are dumped (Roth, 1964). Females are faced with a dichotomy, should they resorb oocytes or should they wait for sperm? To resorb oocytes may prevent loss of resources, but has potential fitness consequences if females then mate within that reproductive cycle, as there are fewer oocytes to fertilize. Logically, females would benefit from resorbing oocytes when males are absent and maintaining oocytes when males are present. Oocyte resorption has been shown to be sensitive to pheromones in the desert locust (*Schistocerca gregaria*), where the scent of a conspecific mature adult male both promotes oocyte growth and inhibits the resorption of oocytes within females (Highnam & Lüsis, 1962; Mahamat *et al.*, 1993). However, the relationship between ovarian physiology and male pheromones has not been explicitly investigated in *N. cinerea*.

Our hypothesis was that females are able to vary their investment in reproduction with the perceived availability of males. We predicted that pheromone exposure would sustain oocyte maintenance for longer by reducing oocyte resorption. Hence, we would find fewer apoptotic oocytes. Second, we predicted that male pheromone would have a gonadotrophic effect, increasing the rate of oocyte maturation. To investigate our predictions we measured the effects of the male pheromone blend on measures of reproduction and somatic investment over time.

Materials and methods

Animal husbandry

Mass colonies were housed under standard rearing conditions of 28°C with 12/12 h reversed light/dark cycle. We removed late instar female nymphs from the mass colonies and created an all-female nymph colony in a 32 × 24 × 10 cm plastic box. We checked the nymph colony daily for females that had completed the adult molt, and we isolated these newly eclosed adults in individual 11 × 11 × 3 cm plastic containers. Females were randomly assigned to an incubator, time, and pheromone treatment. All animals had free access to rat chow and water. We measured pronotum width, a commonly used marker of body size, and eclosion mass of all females. There was no difference in the size or eclosion mass of cockroaches put into different incubator treatments (mass ANOVA, $F_{3,152} = 0.295$, $P = 0.829$; size ANOVA, $F_{3,152} = 1.499$, $P = 0.217$).

Pheromone exposure

As control females must be kept separate from the pheromone, we housed females in separate incubators. In this design we had four incubators to avoid pseudoreplication (Hurlbert, 1984). Two groups were exposed to male pheromone blend in acetone, and the other two were exposed only to acetone in a block design so that we were able to statistically control for the effect of the incubator. Newly eclosed females were given 24 h after molting to allow the exoskeleton to keratinize before the pheromone or sham solution was applied. A 4-mm diameter piece of filter paper was placed in the centre of their box and 250 μL of either pheromone blend or acetone was dropped upon it, this was repeated every 5 days. The male pheromone blend has three components, 2-methylthiazolidine and 4-ethyl-2-methoxyphenol that are genetically coupled and the independent 3-hydroxy-2-butanone (Moore & Moore, 1999). The artificial blend we used consisted of 400 ng/μL 2-methylthiazolidine, 100 ng/μL 4-ethyl-2-methoxyphenol and 300 ng/μL 3-hydroxy-2-butanone in an acetone carrier, as used in previous studies (Moore, 1997; Moore *et al.*, 2001; Moore & Moore, 1999; Moore & Moore, 2003). No pheromone application was done in the vicinity of the control females.

Analysis of reproductive and somatic investment

Nauphoeta cinerea has paired panoistic ovaries consisting of ovarioles connected to a lateral oviduct (Chapman, 1998). Each ovariole consists of a distal germinarium containing oogonia and prefollicular cells and the more proximal vitellarium, where the oocytes undergo vitellogenesis (Chapman, 1998; Moore & Sharma, 2005). In *N. cinerea* the ovariole contains three oocytes containing germinal vesicles, the terminal (T), primary (T-1) and secondary (T-2) oocytes. The terminal oocyte is considerably more matured than the others (Chapman, 1998).

Both the ovary assayed for apoptosis and the other non-stained oocyte were mounted on cavity slides and photographed using a SPOT Insight camera (Diagnostic Instruments, Inc., Stirling Heights, MI, US) with a Leica MZ12.5 dissecting stereomicroscope (Meyer). We counted the number of ovarioles in each ovary and measured the height of all oocytes that contain germinal vesicles (T, T-1 and T-2) from each ovariole in both ovaries and these were measured using Spot Basic version 4.1 (Diagnostic Instruments, Inc.).

To analyze somatic investment, we weighed females at eclosion and dissection to calculate change in mass. At dissection we removed the fat bodies to assay what components of mass were resource stores. To ascertain dry fat body mass we placed fat bodies on foil for which the weight had already been measured and dried for 48 h at 75°C. Foil was then weighed and the dry fat body mass was determined using a UMX2 ultra-microbalance (Mettler-Toledo Ltd., Leicester, UK).

Analysis of oocyte degradation

We dissected females at days 7, 12, 17, and 22. We chose these dates as they are biologically relevant in the process of sexual maturation and reproductive decline: 7 days is the start of oocyte maturation; 12 days is the log growth of oocytes where variance increases (Roth, 1964); and days 17–22 demarcates the period where oocytes are apoptotic (Moore & Sharma, 2005).

One ovary from each individual was dissected and stained using Vybrant Apoptosis Assay kit #4 (Molecular Probes, Invitrogen, Eugene, OR, USA) as described by Moore and Sharma (2005). The dye YO-PRO-1 (green fluorescence) can enter apoptotic cells but is excluded from healthy cells, while propidium iodide (red fluorescence) cannot enter living nor apoptotic cells due to its large molecular size and thus only stains cells which are either necrotic or in the late stages of apoptosis (Moore & Sharma, 2005; Willingham, 1999). Therefore differences in cell membrane permeability can be graded. Healthy oocytes are unstained, apoptotic oocytes are green and necrotic, and oocytes in the late stages of apoptosis are red (Moore & Sharma, 2005). Apoptosis was measured

Table 1 The effects of pheromone application and time (days) on the reproductive and somatic resource physiology of female *Nauphoeta cinerea*.

		F	*d.f.*	*P*
Change in mass	Treatment	0.034	1,149	0.854
	Day	1.226	3,149	0.302
	Treatment × day	1.292	3,149	0.279
% Terminal ooctye apoptosis	Treatment	0.377	1,147	0.54
	Day	9.667	3,147	<0.001
	Treatment × day	0.347	3,147	0.792
% Vitellarium ooctye apoptosis	Treatment	0.000	1,147	1.000
	Day	0.711	3,147	0.547
	Treatment × day	0.000	3,147	1.000
Fat body mass	Treatment	1.480	1,149	0.226
	Day	2.477	3,149	0.063
	Treatment × day	0.885	3,149	0.451
Terminal oocyte volume	Treatment	0.000	1,145	0.988
	Day	24.370	3,145	<0.001
	Treatment × day	0	3,145	1.000
T-1 oocyte volume	Treatment	0.204	1,144	0.652
	Day	36.253	3,144	<0.000 1
	Treatment × day	0.071	3,144	0.975
T-2 oocyte volume	Treatment	0.050	1,142	0.825
	Day	5.393	3,142	0.002
	Treatment × day	0.071	3,142	0.975

in both the mature terminal oocyte in each ovariole and in the non-mature oocytes present in the vitellarium.

Statistics

All ANOVAs were performed using JMP 5.0.1a. All data were blocked by incubator to control for incubator effects.

Results

A summary of all ANOVA statistics is given in Table 1. The passing of time had a significant effect, and over time oocytes grew larger and the number of apoptotic terminal oocytes increased. However, the presence of pheromone did not have any significant effect on ovarian apoptosis, oocyte growth, or fat stores. As non-significant results can also be the result of type II errors we wished to investigate whether it is safe to reject the alternative hypothesis that pheromone had a significant effect on resource distribution. Reterospective power analyses are problematic (Hoenig & Heisey, 2001; Nakagawa & Foster, 2004), so we calculated effect size statistics (r) and the confidence intervals of the mean differences as suggested by Nakagawa and Cuthill (2007), the results of which are summarized in Table 2. When confidence intervals include zero we can be 95% confident that there is no effect (Nakagawa & Cuthill, 2007), and our calculations show that all measures included zero.

Table 2 The effect size and confidence intervals for mean differences on the reproductive and somatic resource physiology of female *Nauphoeta cinerea* between pheromone application treatment and control.

	r	Lower CI	Upper CI
Change in mass	0.013	−0.024 59	0.020 79
Fat body mass	0.426	−8.000 20	0.632 30
% terminal ooctye apoptosis	0.044	−13.593 40	7.715 20
% vitellarium ooctye apoptosis	0.002	−2.178 34	2.231 29
Terminal oocyte volume	0.009	−0.199 27	0.177 37
T-1 oocyte volume	0.044	−0.030 36	0.017 36
T-2 oocyte volume	0.024	−0.016 13	0.011 96

Discussion

Over time oocytes grew larger and the number of apoptotic terminal oocytes increased, which is in agreement with previous work that also shows these trends (Moore & Sharma, 2005; Roth, 1964). However, we found no evidence of male pheromones having any effect on measures of reproductive or somatic resource physiology. Hence, we cannot reject the null hypothesis that female *N. cinerea* have not evolved a mechanism to synchronize investment in oocytes with the presence of male pheromone. Negative results are always difficult to interpret, as ultimately we cannot determine whether female reproductive investment is unresponsive to male pheromone, or is biologically responsive, but not to our methodology, despite the success of the artificial pheromone blend in previous studies (Moore, 1997; Moore & Moore, 1999, 2001, 2003). Ultimately, further work is required before we can eliminate a potential role of male pheromone in female resource physiology dynamics.

One tentative interpretation of this study is that female investment decisions are not sensitive to male pheromones in the first pre-oviposition period. It may be that the aspect of social environment that is important to females in the first pre-oviposition period is mating itself (Wheeler, 1996), which results in an increase in oocyte growth and a decline in oocyte degradation prior to fertilization, which happens 4 days later (Roth, 1964). Previous studies on male pheromones and female reproduction all report effects of male pheromones during embryological development (Moore *et al.*, 2002; Moore & Moore, 2003). Exposure to different components of male pheromone during the embryological period can manipulate the rate at which offspring develop (Moore *et al.*, 2002; Moore & Moore, 2003), the sex ratio of the offspring (Moore *et al.*, 2001), and female lifespan (Moore *et al.*, 2003), whereas in asexual clutches, male pheromone appears to increase clutch size via increasing embryonic viability (Moore & Moore, 2003). Clearly, more work is required to elucidate how social environment, mediated either through direct effects of mating or indirectly through male pheromones, affect female reproductive physiology, and this should prove a fruitful area for future research.

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References

Andux, S. and Ellis, R.E. (2008) Apoptosis maintains oocyte quality in aging *Caenorhabditis elegans* females. *PLoS Genetics*, 4, e10000295.

Apfeld, J. and Kenyon, C. (1999) Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature*, 402, 804–809.

Assad, Y.O.H., Hassanali, A., Torto, B., Mahamat, H., Bashir, N.H.H. and Bashir, S.E.L. (1997) Effects of fifth-instar volatiles on sexual maturation of adult desert locust *Schistocerca gregaria*. *Journal of Chemical Ecology*, 23, 1373–1386.

Barrett, E.L.B., Moore, A.J. and Moore, P.J. (2009) Diet and social conditions during sexual maturation have unpredictable influences on female life history trade-offs. *Journal of Evolutionary Biology*, 22, 571–581.

Barrett, E.L.B., Moore, A.J. and Moore, P.J. (In Press) A potential function for oocyte apoptosis in *Nauphoeta cinerea*. *Physiological Entomology*.

Chapman, R.F. (1998) *The Insects – Structure and Function*. Cambridge, UK, Cambridge University Press.

Corley, L.S., Blankenship, J.R. and Moore, A.J. (2001) Genetic variation and asexual reproduction in the facultatively parthenogenetic cockroach *Nauphoeta cinerea:* implications for the evolution of sex. *Journal of Evolutionary Biology*, 14, 68–74.

Corley, L.S., Blankenship, J.R., Moore, A.J. and Moore, P.J. (1999) Developmental constraints on the mode of reproduction in the facultatively parthenogenetic cockroach *Nauphoeta cinerea*. *Evolution and Development*, 1, 90–99.

Corley, L.S. and Moore, A.J. (1999) Fitness of alternative modes of reproduction: developmental constraints and the evolutionary maintenance of sex. *Proceedings of the Royal Society Biological Sciences Series B*, 266, 471–476.

Fissore, R.A., Kurokawa, M., Knott, J., Zhang, M. and Smyth, J. (2002) Mechanisms underlying oocyte activation and postovulatory ageing. *Reproduction (Cambridge)*, 124, 745–754.

Highnam, K.C. and Lüsis, O. (1962) The influence of mature males on the neurosecretory control of ovarian development in the desert locust. *Quarterly Journal of Microscopial Science*, 103, 73–78.

Hill, E.M. and Low, B.S. (1992) Contemporary abortion patterns: a life history approach. *Ethology and Sociobiology*, 13, 35–48.

Hoenig, J.M. and Heisey, D.M. (2001) The abuse of power: the pervasive fallacy of power calculations for data analysis. *American Statistician*, 55, 1–6.

Hurlbert, S.H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54, 187–211.

Lynch, M. and Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sunderland, Sinauer.

Mahamat, H., Hassanali, A., Odongo, H., Torto, B. and El-Badhir, E.-S. (1993) Studies on the maturation-accelerating pheromone of the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae). *Chemoecology*, 4, 159–164.

Marcillac, F. and Ferveur, J.-F. (2007) A set of female pheromones affects reproduction before, during and after mating in *Drosophila*. *Journal of Experimental Biology*, 207, 3927–3933.

Moore, A.J. (1997) The evolution of social signals: morphological, functional, and genetic integration of the sex pheromone in *Nauphoeta cinerea*. *Evolution*, 51, 1920–1928.

Moore, A.J., Gowaty, P.A. and Moore, P.J. (2003) Females avoid manipulative males and live longer. *Journal of Evolutionary Biology*, 16, 523–530.

Moore, A.J., Gowaty, P.A., Wallin, W.G. and Moore, P.J. (2001) Sexual conflict and the evolution of female mate choice and male social dominance. *Proceedings of the Royal Society Biological Sciences Series B*, 268, 517–523.

Moore, A.J., Haynes, K.F., Preziosi, R.F. and Moore, P.J. (2002) The evolution of interacting phenotypes: genetics and evolution of social dominance. *American Naturalist*, 160, S186–S197.

Moore, A.J. and Moore, P.J. (1999) Balancing sexual selection through opposing mate choice and male competition. *Proceedings of the Royal Society Biological Sciences Series B*, 266, 711–716.

Moore, A.J., Reagan, N.L. and Haynes, K.F. (1995) Conditional signalling strategies: effects of ontogeny, social experience and social status on the pheromonal signal of male cockroaches. *Animal Behaviour*, 50, 191–202.

Moore, P.J., Harris, W.E. and Moore, A.J. (2007) The cost of keeping eggs fresh: quantitative genetic variation in females that mate late relative to sexual maturation. *American Naturalist*, 169, 311–322.

Moore, P.J. and Moore, A.J. (2001) Reproductive aging and mating: The ticking of the biological clock in female cockroaches. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 9171–9176.

Moore, P.J. and Moore, A.J. (2003) Developmental flexibility and the effect of social environment on fertility and fecundity in parthenogenetic reproduction. *Evolution and Development*, 5, 163–168.

Moore, P.J. and Sharma, S. (2005) A delay in age at first mating results in the loss of future reproductive potential via apoptosis. *Evolution and Development*, 7, 216–222.

Nakagawa, S. and Cuthill, I.C. (2007) Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Review*, 82, 591–605.

Nakagawa, S. and Foster, T.M. (2004) The case against reterospective statistical power analyses with an introduction to power analysis. *Acta Ethologica*, 7, 103–108.

Ricklefs, R.E. and Wikelski, M. (2002) The physiology/life-history nexus. *Trends in Ecology and Evolution*, 17, 462–468.

Ritchie, M.G. (2000) The inheritance of female preference functions in a mate recognition system. *Proceedings of the Royal Society Biological Sciences Series B*, 267, 327–332.

Roff, D.A. (1992) *The Evolution of Life Histories: Theory and Analysis*. New York, Springer.

Roth, L.M. (1964) Control of reproduction in female cockroaches with special reference to *Nauphoeta cinerea* – I. First pre-oviposition period. *Journal of Insect Physiology*, 10, 915–945.

Schluter, D., Price, T.D. and Rowe, L. (1991) Conflicting selection pressures and life history trade-offs. *Proceedings of the Royal Society Biological Sciences Series B*, 246, 11–17.

Sreng, L. (1990) Seducin, male sex pheromone of the cockroach *Nauphoeta cinerea*: Isolation, identification, and bioassay. *Journal of Chemical Ecology*, 16, 2899–2912.

Sreng, L. (2003) Sensory asymmetries in the olfactory system underlie sexual pheromone communication in the cockroach *Nauphoeta cinerea*. *Neuroscience Letters*, 351, 141–144.

Stearns, S.C. (1992) *The Evolution of Life Histories*. New York, Oxford University Press.

Stern, K. and McClintock, M.K. (1998) Regulation of ovulation by human pheromones. *Nature*, 392, 177–179.

Tanaka, E.D., Schmidt Capella, I.C. and Hartfelder, K. (2006) Cell death in the germline – mechanisms and consequences for reproductive plasticity in social bees. *Brazilian Journal of Morphological Sciences*, 23, 15–26.

Tawfik, A.L., Treiblmayr, K., Hassanali, A. and Osir, E.O. (2000) Time-course haemolymph juvenile hormone titres in solitarious and gregarious adults of *Schistocerca gregaria*, and their relation to pheromone emission, CA volumetric changes and oocyte growth. *Journal of Insect Physiology*, 46, 1143–1150.

Vasi, F., Travisano, M. and Lenski, R.E. (1994) Long-term experimental evolution in *Escherichia coli*. II. changes in life-history traits during adaptation to a seasonal environment. *American Naturalist*, 144, 432–456.

Wedekind, C., Seebeck, T., Bettens, F. and Paepke, A.J. (1995) MHC-Dependent mate preferences in humans. *Proceedings of the Royal Society Biological Sciences Series B*, 260, 245–249.

Wheeler, D. (1996) The role of nourishment in oogenesis. *Annual Review of Entomology*, 41, 407–431.

Willingham, M.C. (1999) Cytochemical methods for the detection of apoptosis. *The Journal of Histochemistry and Cytochemistry*, 47, 1101–1109.

Wyatt, T.D. (2003) *Pheromones and Animal Behaviour*. Cambridge University Press, Cambridge, UK.

Ylönen, H. and Ronkainen, H. (1994) Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. *Evolutionary Ecology*, 8, 658–666.

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