

Recognition of nestmate eggs in the ant *Formica fusca* is based on queen derived cues

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Abstract Inclusive fitness benefits depend on recognizing the right individuals to interact with. Social insect nests protect themselves from non-kin intruders through nestmate recognition based on chemical cues. The recognition cues on adult individuals are from a mixture of genetic and environmental sources, but the ontogeny and use of recognition cues on eggs has not been previously assessed. We studied recognition by workers of eggs that were either nestmates or non-nestmates, and the ontogeny of recognition cues on eggs in the ant *Formica fusca*, a species with precise egg recognition abilities. Workers were able to discriminate among freshly laid eggs with no nest derived cues on them, and the egg surface chemicals varied among nests in these eggs, suggesting that queen derived cues are used in nestmate recognition. The results are discussed in the light of their implications on deceptive social parasite strategies and within colony conflicts [Current Zoology 60 (1): 131–136, 2014].

Keywords Nestmate recognition, Social insects, Social parasitism, *Formica fusca*, Cuticular hydrocarbons

Recognition is a fundamental aspect of social behaviour (D'Ettorre and Hughes, 2008). The inclusive fitness of individuals may depend, for example, on recognising the right species or kind of individual to mate with, recognising individuals in a social hierarchy, or on being able to exclude intruders from a social group. However, cheating strategies where individuals gain benefits from deception, so that their identity is mistaken, are also commonplace (Ghoul et al., in press). For example, cuckoos benefit from the misdirected parental effort of the host, who has reduced fitness through loss of own brood (Kilner and Langmore, 2011). Similarly, brood guarding males lose paternity through the sneaker tactics of female-mimicking males, in several species of fish (Taborsky, 2008).

Recognition is especially important in kin-selected social contexts where benefits of co-operation are shared among kin. Recognition of family members can be based on two broad classes of cues. First, discrimination can be based on endogenous cues produced by the individuals that carry them, if each family member innately e.g. due to genetic similarity produces more similar cues than individuals in other families. For example, marine invertebrates such as colonial ascidians reject non-relatives based on similarity at a highly variable fusion/histocompatibility locus (Grosberg and Quinn, 1986).

Second, cues can also be environmentally acquired, or exogenous, if each family shares a distinct environmental source of cues. For example, in social insects such as *Polistes* paper wasps, odours acquired from the nest material are crucial in giving young individuals a distinct nest specific cue profile (Gamboa, 2004).

In social insects, recognising family members guarantees inclusive fitness benefits of non-reproductive worker strategies. However, the shared resources and cooperative efforts of families are threatened by both con-specifics, such as robbers in honeybees (Downs and Ratnieks, 1999), territorial invaders in ants (Hölldobler and Lumsden, 1980), and non-conspecifics such as socially parasitic queens who take over or exploit a host colony (Lenoir et al., 2001). Family recognition in social insects occurs predominantly through recognising nestmates rather than directly assessing relatedness (van Zweden and D'Ettorre, 2010). Nestmate recognition between adult individuals has been studied in a wide range of species, and is based on both environmentally acquired and individually produced cues (van Zweden and D'Ettorre, 2010; Martin et al., in press).

However, there are only few studies of discrimination between nestmate and non-nestmate eggs, mainly from *Formica* ants (Lorenzi and Filippone, 2000; Johnson et al., 2005; Helanterä et al., 2007; Helanterä

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and Sundström, 2007; Helanterä and Ratnieks, 2009; Meunier et al., 2010; Chernenko et al., 2011; Meunier et al., 2011) and these studies have not assessed the ontogeny, more specifically the relative roles of genetic and environmental sources, of cues used. The ontogeny of cues determines how vulnerable egg recognition is to deception. If recognition is based on endogenous cues that are on the eggs when they are laid, whether derived from the queen or produced by the egg, then discrimination against intruders may be possible even when they are sharing the environment. If cues are exogenous, that is acquired from the environment after the eggs have been laid, discriminating among eggs laid in a shared environment by different individuals is not possible. This would mean that discrimination against eggs of non-kin individuals who share the colony environment, such as social parasite queens that have invaded the colony (Lenoir et al., 2001), could be impossible. Social parasite queens may be able to match their cuticular odour to the profile of their host through camouflage very closely (Guillem et al., 2014), which suggests that similar processes could work with eggs as well.

We studied the source of cues used in nestmate recognition of eggs by workers of the ant *Formica fusca*, a species where both nestmate recognition of conspecific eggs (Helanterä et al., 2007; Helanterä and Sundström, 2007; Helanterä and Ratnieks, 2009) and discrimination of eggs of different queens within nests (Ozan et al., 2013) have been demonstrated, although these abilities seem to be absent in populations in the UK (Martin et al., 2011). We compared acceptance of eggs of nestmate and non-nestmate queens laid in petri dishes without contact to nest material or nestmates (only queen-derived cues present) versus eggs taken from the colony environment (cues from the nest environment also present). The surface hydrocarbons of eggs from the two sources were analysed to complement the experimental results.

1 Materials and Methods

1.1 The bioassay

Nine single-queen laboratory colonies from a Finnish *Formica fusca* population were set up in April 2006 as detailed in Helanterä et al. (2007). When egg laying resumed in the spring (eggs are not laid in winter), egg acceptance bioassays were carried out following Helanterä et al. (2007). In a bioassay, workers on a neutral plaster floored arena are offered eggs on a piece of acetate sheet, and eggs picked up and moved to a pile or carried around by workers in 2 hours are classified as accepted. The person counting the collected eggs after

the trial was blind to the treatment. Bioassays were carried out using four different types of queen-laid eggs: nestmate queen (bioassays were carried out using 5 different discriminator colonies) or non-nestmate queen ($n=9$ discriminator colonies) with only queen-derived cues; nestmate ($n=8$) or non-nestmate ($n=8$) queen with both queen derived and nest-environment cues. Eggs that carried both queen- and environmentally derived cues (from now on “Nestbox eggs”) were taken from the laboratory nestboxes, and were up to one week old, but usually younger since eggs were taken into experiments every two days, and most eggs were taken. They had thus had ample time to acquire cues from workers and nest material. Eggs with queen-derived cues only (“petri dish eggs”) were obtained from queens that had been separated from the colony for 12 hours on a plastic 90 mm diameter petri dish, where they laid the eggs. A queen taken from a mature colony of *F. fusca* on her own in a petri dish does not handle the eggs once she has laid them (personal observations by the authors). Thus, at the time of the bioassay, the eggs collected from the petri dishes only had the cues on them that they already had when laid. If eggs carry queen derived cues relevant to nestmate recognition when laid, we predict that workers will be able to discriminate between eggs laid by different queens in petri dishes. If eggs only acquire cues from the nest environment and workers, we predict that workers will accept eggs laid in petri dishes by both nestmate and non-nestmate queens, but discriminate over non-nestmate eggs taken from the colony environment, as in previous studies.

1.2 Statistical analyses of behavioural data

Egg acceptance (counts of accepted and rejected eggs) was analysed by a GLMM with a binomial error structure (command `glmer` in R library `lme4`), with nestmate status (Nestmate vs Non-nestmate) and egg origin (Nestbox eggs vs Petri dish eggs) as fixed factors. Egg source nest ID and discriminator nest ID were included as random factors to account for using the same nests in several factor combinations, and on a few occasions (1 in Non-nestmate/petri-dish, 7 in Non-nestmate / Nestbox, 5 in Nestmate / Nestbox) several times in the same combination. Confidence intervals of effect size estimates are given as 1.96 time the standard error as suggested by Nakagawa and Cuthill (2007).

1.3 Chemical analysis of egg surface hydrocarbons

Each egg sample consisted of a pool of either 10 nest box or petri dish eggs for a single queen. A pooled sample was used to ensure sufficient material was extracted for analysis. Each pool of 10 eggs was placed into a

glass vial with 30 µl of HPLC grade hexane. After 10 minutes, the hexane was removed and placed into another clean vial and evaporated. Vials with the dried extract were then sealed and stored at 5°C. Just prior to analysis 30 µl of hexane was added to the vials and the sample analysed on a HP 6890 GC (equipped with a HP-5MS column; length: 30 m; ID: 0.25 mm; film thickness: 0.25 µm) connected to a HP5973 MSD (quadrupole mass spectrometer with 70-eV electron impact ionization). Samples were injected in the splitless mode and the oven was programmed from 70°C to 200°C at 40°C/min and then from 200°C to 320°C at 25°C/min and held for 2 min at 320°C. Helium was used as carrier gas, at a constant flow rate of 1.0 ml min⁻¹. CHCs were characterized by the use of standard Mass Spectrum databases, diagnostic ions and their Kovats indices.

1.4 Statistical analyses of chemical data

The peak areas were standardised before statistical analyses according to the formula $Z_{i,j} = \ln[Y_{i,j}/g(Y_j)]$, where $Y_{i,j}$ is the area of peak i for ant j , $g(Y_j)$ is the geometric mean of the areas of all peaks for ant j , and $Z_{i,j}$ is the standardised area of peak i for ant j (Aitchison, 1986). Only peaks consistently present in all individuals were used. Chemical differences among nests, and among egg origin treatments were studied with a 2*2 non-parametric permutational analogue of MANOVA (“adonis” in R package “vegan” (Oksanen et al., 2013)), with nests and egg origin treatments as explanatory variables. In addition to the 2*2 adonis analyses, we investigated the amount of variation among nests separately for both egg ori-

gin treatments.

2 Results

Workers accepted more eggs laid by nestmate queens whether from the petri dish or nest box (Fig. 1, acceptance 74%, $SD = 31\%$, and 59%, $SD = 32\%$, respectively) than the corresponding eggs from non-nestmate queens (4%, $SD = 7\%$, and 3%, $SD = 6\%$ respectively). Only nestmate status, not the types of cues, affected acceptance of eggs (Nestmate status $z = 10.0$, $P < 0.001$, effect size [95% CI] = 6.0 [4.8 – 7.2], egg origin $z = -0.07$, $P = 0.94$, effect size [95% CI] = -0.06 [-1.7 – 1.6] nestmate status * egg origin $z = 0.54$, $P = 0.6$, effect size [95% CI] = 0.5 [-1.4 – 2.4]).

Eggs taken from petri dishes contained the same compounds as the eggs taken from the nest boxes (example profiles of eggs in Fig. 2), with only quantitative

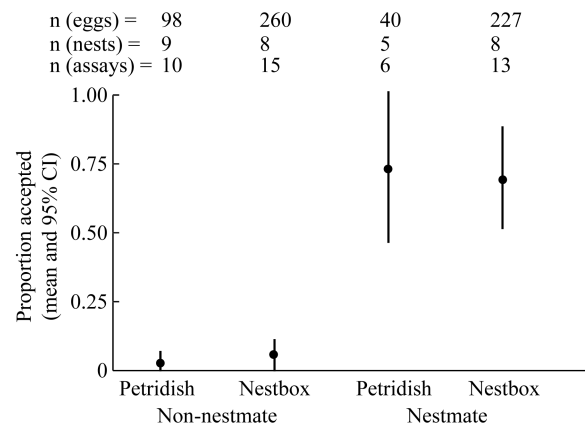


Fig. 1 Acceptance of eggs (circles = means, bars = 95% CI, calculated from the n of assays) from different sources

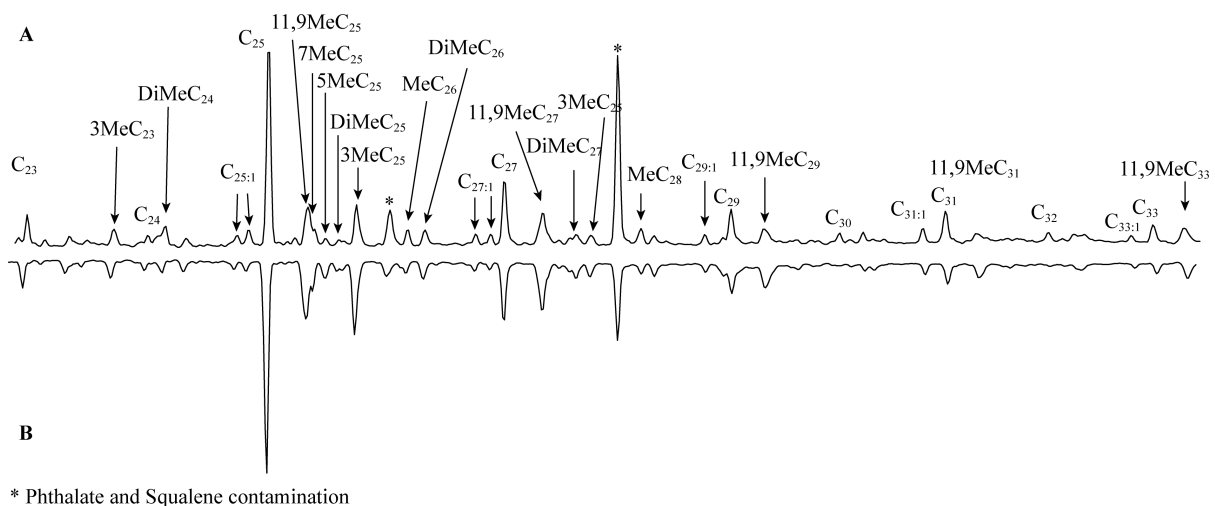


Fig. 2 Typical surface hydrocarbon profiles of eggs (total ion chromatograms) from one of the colonies showing the a) a pooled sample of 10 eggs laid on petri dish and b) a pooled sample of 10 eggs taken from the nest, which is inverted to aid comparisons to be made

The major compounds, where possible have been identified. Alkanes = C_n with n representing the carbon chain length, alkenes = C_n:₁, Methylalkanes = nMeC_n and dimethylalkanes = DiMeC_n. The peaks identified as a contamination (*) were not included in the analysis.

differences in compound abundance. This was the case for each nest. Eggs were chemically different in the proportions of compounds both among nests ($F_{3,20} = 8.2$, $R^2 = 35\%$, $P < 0.001$), and among treatments ($F_{1,20} = 17.2$, $R^2 = 25\%$, $P < 0.001$, for the interaction term $F_{3,20} = 2.7$, $R^2 = 12\%$, $P = 0.003$). When analysed separately, the among nests differences in egg chemistry were found in both egg origin treatments (petri dish eggs $F_{3,9} = 2.6$, $R^2 = 47\%$, $P = 0.02$, Nestbox eggs $F_{3,11} = 90.0$, $R^2 = 71\%$, $P < 0.001$). The effect sizes for nest differences were not significantly different from each other (test for heterogeneity of effect sizes $P = 0.8$).

3 Discussion

This study gives clear evidence that workers of *F. fusca* are able to discriminate between nestmate and non-nestmate eggs based on the cues on freshly laid eggs. Our chemical analyses support the behavioural observations by showing that young eggs contain a nest specific chemical profile when laid. While statistically significant chemical changes do occur after the eggs have spent time in the nest environment, they neither prevent nor enhance the use of cues by workers.

Such discrimination of eggs is not a general pattern in social insects. For example, honeybees (Ratnieks and Visscher, 1989), common wasps *Vespula vulgaris* (Foster and Ratnieks, 2001), and carpenter ants *Camponotus floridanus* (Endler et al., 2004) do not have similar egg recognition, but accept eggs laid by non-nestmate queens. In Finland where the study was conducted *F. fusca* is a host to several species of temporary social parasite queens of the same genus that invade the colonies and make the host workers rear parasite brood. Thus, *F. fusca* may sometimes face heterospecific eggs in their colonies. While more comprehensive comparisons among parasitised and parasite free populations and species are needed to assess the role of the parasitic deceivers in driving the precise recognition, and the maintenance of cue diversity, the lack of egg recognition and loss of chemical diversity in parasite free UK *F. fusca* populations (Martin et al., 2011) suggests that such a link could exist.

Presence of informative queen-derived cues on newly laid eggs, and the ability of workers to assess them have implications for the tactics social parasites may use in order to get their eggs reared by the hosts. First, parasite queens may lay eggs that lack the key cues workers use in discriminating against aliens (Cervo et al., 2008; Martin et al., 2008c). Eggs that lack the relevant recognition cues may be accepted by workers if they reject

eggs based on presence of unfamiliar cues (Couvillon and Ratnieks, 2008; Guerrieri et al., 2009; Ratnieks et al., 2011), rather than the lack of familiar, desirable cues. However, the fact that *F. fusca* workers are able to discriminate against eggs laid by queens of socially parasitic *Formica truncorum* and *Formica aquilonia* (Chernenko et al., 2011; Chernenko et al., 2013) does not support this scenario. The results of this study also do not support the idea that rejection is due to simple presence or absence of unfamiliar cues since all the *F. fusca* colonies had very similar chemical profiles that mainly varied quantitatively not qualitatively, as shown in other ant species as well (Martin et al., 2012b). Furthermore, analyses of eggs laid by queens of *Formica aquilonia* (Schultner et al., 2013), *F. lugubris* and three species of the *F. exsecta* group (SJM, HH, unpublished data) which are other potential social parasites, show that their eggs are not chemically insignificant i.e. have a rich chemical profile similar to that of the adult individuals of the species.

Second, parasite eggs may be accepted if they bear cues similar to those of the host. However, in many situations the chemistry of parasitic species as a whole does not match that of the hosts (Martin et al., 2008b). For example, the eggs of *F. aquilonia*, a potential social parasite, contain a large number of compounds not found on *F. fusca* eggs (Schultner et al., 2013). However, it is possible that parasite queens are able to acquire the relevant profile from the nest environment, because a parasite queen that survives in the colony until egg laying is fed by the workers, and becomes integrated into the colony. Recent evidence has shown that in some ant social parasites, mimicry down to the colony level does occur in the odour profiles of the queens (Guillem et al., 2014), but studies of egg odours in such nests are still lacking.

The odour profiles of eggs also varied between eggs collected from nestboxes and petri dishes. While our experimental setup does not allow us to exclude effects caused by the plastic petri dish, it is likely that handling by workers does change egg surface odours significantly, as they help unify any small differences to produce the colony odour shared by all nestmates (Helanterä et al., 2011, Martin et al., 2012a). No changes in the presence or absence of compounds were observed, which fits with the observation that odour profiles on adult individuals of *F. fusca* consists largely of the same compounds as the egg profiles (Martin et al., 2008a, Martin et al., 2011).

The source of recognition cues on eggs also has im-

plications for within colony conflict. Queen derived recognition cues on eggs are necessary for within colony discrimination, such as favoring eggs laid by certain queens in multi-queen colonies. Such discrimination has been demonstrated in *Formica fusca* (Ozan et al., 2013), but the precise ontogeny of queen derived eggs remains uncharted. Discrimination among eggs laid in the same nest environment suggests that cues are not purely exogenous. However, the extent to which the variation in queen derived cues reflects e.g. kinship, and would thus underlie nepotism (Hannonen and Sundström, 2003) is unclear. As all the queens in our study were overwintered mature queens, it is also unlikely that our results would reflect differences in fertility signals on eggs, as in *Camponotus floridanus* (Moore and Liebig, 2010). To separate the genetic (both direct and sib-social genetic effects) (Linksvayer, 2006; Van Zweden et al., 2010) and environmental components of egg recognition cues, future studies should assess chemical differences between eggs within nests, ideally also in cross fostering experiments where nestmate and non-nestmate queens are exposed to different colony environments, including the workers attending the queens.

Availability of information has been proposed to be one of the universal constraints of adaptation (West and Sheldon, 2002). While for evolution of morphological traits the constraints of physics, development and genetic architecture of traits play a large role, for social recognition behaviours origin of available recognition cues is a major constraint of adaptation. Furthermore, not just the use of information, but also the recognition cues themselves are subject to evolutionary forces (Crozier, 1986; Ratnieks et al., 2007; Rousset and Roze, 2007; Holman et al., 2013), and whether cues are genetically or environmentally derived plays a large role in the maintenance of informative cues. Thus, teasing apart different sources of cues will help us understand both who has power to win within group conflicts (Beekman et al., 2003), but also how the cues and their use may evolve and what implications this has for interspecific relationships where one party deceives the sensory systems of the other.

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References

Aitchison J, 1986. The statistical analysis of compositional data. London: Chapman & Hall.
Beekman M, Komdeur J, Ratnieks FLW, 2003. Reproductive

conflict in social animals: Who has power? Trends Ecol. Evol. 6: 277–282.
Cervo R, Dani FR, Cotoneschi C, Scala C, Lotti I et al., 2008. Why are larvae of the social parasite wasp *Polistes sulcifer* not removed from the host nest? Behav. Ecol. Sociobiol. 62: 1319–1331.
Chernenko A, Helanterä H, Sundström L, 2011. Egg recognition and social parasitism in *Formica* ants. Ethology 117: 1081–1092.
Chernenko A, Vidal-Garcia M, Helanterä H, Sundström L, 2013. Colony take-over and brood survival in temporary social parasites of the ant genus *Formica*. Behav. Ecol. Sociobiol. 67: 727–735.
Couvillon MJ, Ratnieks FLW, 2008. Odour transfer in stingless bee marmelada *Frieseomelitta varia* demonstrates that entrance guards use an "undesirable-absent" recognition system. Behav. Ecol. Sociobiol. 62: 1099–1105.
Crozier RH, 1986. Genetic clonal recognition abilities in marine-invertebrates must be maintained by selection for something else. Evolution 40: 1100–1101.
D'Ettorre P, Hughes DP, 2008. Sociobiology of Communication: An Interdisciplinary Perspective. Oxford: Oxford University Press.
Downs SG, Ratnieks FLW, 1999. Recognition of conspecifics by honeybee guards uses nonheritable cues acquired in the adult stage. Anim. Behav. 58: 643–648.
Endler A, Liebig J, Schmitt T, Parker JE, Jones Gr et al., 2004. Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proc. Natl. Acad. Sci. U. S. A. 101: 2945–2950.
Foster KR, Ratnieks FLW, 2001. Convergent evolution of worker policing by egg eating in the honeybee and common wasp. Proceedings of the Royal Society of London Series B-Biological Sciences 268:169–174.
Gamboa GJ, 2004. Kin recognition in eusocial wasps. Ann. Zool. Fenn. 41: 789–808.
Ghoul M, Griffin AS, West SA, in press. Towards an evolutionary definition of cheating. Evolution.
Grosberg RK, Quinn JF, 1986. The genetic-control and consequences of kin recognition by the larvae of a colonial marine invertebrate. Nature 322: 456–459.
Guerrieri FJ, Nehring V, Jörgensen CG, Nielsen J, Galizia CG et al., 2009. Ants recognize foes and not friends. Proceedings of the Royal Society B: Biological Sciences 276: 2461–2468.
Guillem RM, Drijfhout FP, Martin SJ, 2014. Chemical deception among ant social parasites. Current Zoology 60: – .
Hannonen M, Sundström L, 2003. Sociobiology: Worker nepotism among polygynous ants. Nature 421: 910–910.
Helanterä H, Lee YR, Drijfhout FP, Martin SJ, 2011. Genetic diversity, colony chemical phenotype, and nest mate recognition in the ant *Formica fusca*. Behav. Ecol. 22: 710–716.
Helanterä H, Martin SJ, Ratnieks FLW, 2007. Prior experience with eggs laid by non-nestmate queens induces egg acceptance errors in ant workers. Behav. Ecol. Sociobiol. 62: 223–228.
Helanterä H, Ratnieks FLW, 2009. Two independent mechanisms of egg recognition in worker *Formica fusca* ants. Behav. Ecol. Sociobiol. 63: 573–580.
Helanterä H, Sundström L, 2007. Worker policing and nest mate recognition in the ant *Formica fusca*. Behav. Ecol. Sociobiol.

- 61: 1143–1149.
- Holman L, van Zweden J, Linksvayer T, d'Ettorre P, 2013. Crozier's paradox revisited: maintenance of genetic recognition systems by disassortative mating. *BMC Evol. Biol.* 13: 211.
- Hölldobler B, Lumsden CJ, 1980. Territorial strategies in ants. *Science* 210: 732–739.
- Johnson CA, H Topoff, Vander Meer RK, Lavine B, 2005. Do these eggs smell funny to you? An experimental study of egg discrimination by hosts of the social parasite *Polyergus breviceps* (Hymenoptera : Formicidae). *Behav. Ecol. Sociobiol.* 57: 245–255.
- Kilner RM, Langmore NE, 2011. Cuckoos versus hosts in insects and birds: Adaptations, counter-adaptations and outcomes. *Biological Reviews* 86: 836–852.
- Lenoir A, D'Ettorre P, Errard C, Hefetz A, 2001. Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.* 46: 573–599.
- Linksvayer TA, 2006. Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* 60: 2552–2561.
- Lorenzi MC, Filippone F, 2000. Opportunistic discrimination of alien eggs by social wasps (*Polistes biglumis*, Hymenoptera Vespidae): A defense against social parasitism? *Behav. Ecol. Sociobiol.* 48:402–406.
- Martin SJ, Helanterä H, Drijfhout FP, 2008a. Colony-specific hydrocarbons identify nest mates in two species of Formica ant. *J. Chem. Ecol.* 34:1072–1080.
- Martin SJ, Helanterä H, Drijfhout FP, 2008b. Evolution of species-specific cuticular hydrocarbon patterns in Formica ants. *Biol. J. Linn. Soc.* 95:131–140.
- Martin SJ, Helanterä H, Drijfhout FP, 2011. Is parasite pressure a driver of chemical cue diversity in ants? *Proceedings of the Royal Society B: Biological Sciences* 278: 496–503.
- Martin SJ, Takahashi JJ, Ono M, Drijfhout FP, 2008c. Is the social parasite *Vespa dybowskii* using chemical transparency to get her eggs accepted? *J. Insect Physiol.* 54: 700–707.
- Martin SJ, Trontti K, Shemilt S, Drijfhout F, Butlin R et al., 2012a. Weak patriline effects are present in the cuticular hydrocarbon profiles of isolated *Formica exsecta* ants but they disappear in the colony environment. *Ecology and Evolutionary Biology* 2: 2333–2346.
- Martin SJ, Vitikainen E, Drijfhout F, Jackson DE, 2012b. Conspecific ant aggression is correlated with chemical distance, but not with genetic or spatial distance. *Behav. Genet.* 42: 323–331.
- Martin SJ, Vitikainen E, Shemilt S, Drijfhout FP, Sundström L, in press. Sources of variation in cuticular hydrocarbons in the ant *Formica exsecta*? *J. Chem. Ecol.*
- Meunier J, Delaplace L, Chapuisat M, 2010. Reproductive conflicts and egg discrimination in a socially polymorphic ant. *Behav. Ecol. Sociobiol.* 64: 1655–1663.
- Meunier J, Delémont O, Lucas C, 2011. Recognition in ants: Social origin matters. *PLoS ONE* 6:e19347.
- Moore D, Liebig J, 2010. Mechanisms of social regulation change across colony development in an ant. *BMC Evol. Biol.* 10: 328.
- Nakagawa S, Cuthill IC, 2007. Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews* 82: 591–605.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR et al., 2013. vegan: Community Ecology Package. R package version 2.0-7.
- Ozan M, Helanterä H, Sundström L, 2013. The value of oviposition timing, queen presence and kinship in a social insect. *Proceedings of the Royal Society B: Biological Sciences* 280.
- Ratnieks FLW, Helanterä H, Foster KR, 2007. Are mistakes inevitable? Sex allocation specialization by workers can reduce the genetic information needed to assess queen mating frequency. *J. Theor. Biol.* 244: 470–477.
- Ratnieks FLW, Karcher MH, Firth V, Parks D, Richards A et al., 2011. Acceptance by honey bee guards of non-nestmates is not increased by treatment with nestmate odours. *Ethology* 117: 655–663.
- Ratnieks FLW, Visscher PK, 1989. Worker policing in the honeybee. *Nature* 342: 796–797.
- Rousset F, Roze D, 2007. Constraints on the origin and maintenance of genetic kin recognition. *Evolution* 61: 2320–2330.
- Schultner E, d'Ettorre P, Helanterä H, 2013. Social conflict in ant larvae: Egg cannibalism occurs mainly in males and larvae prefer alien eggs. *Behav. Ecol.* 24: 1306–1311.
- Taborsky M, 2008. Alternative reproductive tactics in fish. In: Oliveria R, Taborsky M, Brockmann HJ ed. *Alternative Reproductive Tactics: An Integrative Approach*. Cambridge: Cambridge University Press, 251–299.
- Van Zweden JS, Brask JB, Christensen JH, Boomsma JJ, Linksvayer TA et al., 2010. Blending of heritable recognition cues among ant nestmates creates distinct colony gestalt odours but prevents within-colony nepotism. *J. Evol. Biol.* 23: 498–508.
- van Zweden JS, D'Ettorre P, 2010. Nestmate recognition in social insects and the role of hydrocarbons. In: Blomquist GJ, Bagneres A ed. *Insect Hydrocarbons: Biology, Biochemistry and Chemical Ecology*. Cambridge: Cambridge University Press, 222–243.
- West SA, Sheldon BC, 2002. Constraints in the evolution of sex ratio adjustment. *Science* 295: 1685–1688.