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Pheromones

Exploitation of gut bacteria in the locust

The congregation of locusts into vast swarms can cause crop devastation of biblical proportions[1]. Here we show that guaiacol, a key component of a pheromone derived from locust faecal pellets that promotes the aggregation of locusts[2–5], is produced by bacteria in the locust gut. This adaptation by an insect to exploit a common metabolite produced by indigenous gut bacteria has wide implications for our appreciation of the role of the gut microbiota in insects.

Guaiacol (2-methoxyphenol) and phenol are volatile compounds that are both released from the faecal pellets of conventionally reared larval and mature adult desert locusts, *Schistocerca gregaria* (Table 1). Guaiacol production from locusts has previously been attributed to the insect itself. However, desert locusts have a large but relatively simple gut bacterial biota[6] which comprises bacterial species acquired from their environment and located in particular in the lining of the hindgut, where faecal pellets form.

We investigated the possible involvement of the gut biota in the production of guaiacol by rearing locusts from surface-sterilized eggs in a sterile isolator system and establishing a breeding colony of axenic (germ-free) locusts by feeding them γ-irradiated freeze-dried grass and bran[7]. Faecal pellets from axenic locusts smelled markedly different from those from locusts with a normal gut biota. Chemical analysis revealed that the difference in odour was due to the absence of guaiacol and low levels of phenol in volatiles released from the germ-free faecal pellets.

We detected guaiacol and phenol in volatiles from the faecal pellets of monoassociated fifth-instar larvae, immature and mature adults carrying a single bacterial species *Pantoea* (=*Enterobacter*) *agglomerans*, a prominent member of the locust-gut biota[6], and reared on the γ-irradiated diet (Table 1). The smaller amount of volatile phenolics released from young-adult monoassociated insects in comparison with other stages correlates with the lower numbers of bacteria in the gut of newly moulted locusts[6]. These results show that guaiacol originates from the gut bacteria. This finding is supported by experiments demonstrating that three different species of bacteria (including *P. agglomerans*) from the locust gut can produce guaiacol directly from axenic faecal pellets *in vitro*.

The precursor for guaiacol synthesis in faecal pellets must either be a component of plant material or an excretory product of the insect itself. The former is most likely, as the amount of guaiacol produced depends on the diet: more guaiacol was produced by normal locusts fed on fresh wheat seedlings than by those fed freeze-dried γ-irradiated grass. Incubation of locust food with bacteria generated only trace amounts of guaiacol or phenol (data not shown), indicating that digestion of the plant material in the locust gut is required for production of guaiacol by the bacteria.

The most likely precursor for guaiacol synthesis is lignin-derived vanillic acid (4-hydroxy-3-methoxybenzoic acid), which is found in the faeces of both axenic and normal locusts[8]. Microbial transformation of vanillic acid to guaiacol requires a decarboxylation step[9]. Consistent with this, we found guaiacol released by all three species of bacteria from glucose/peptone broth cultures containing vanillic acid (data not shown). Furthermore, faeces from conventionally reared insects fed filter paper impregnated with vanillic acid solution yielded large amounts of guaiacol (Table 1).

The gut bacteria of locusts, as in many other insects, are considered to be either commensal or facultatively pathogenic[10], and therefore to have little effect on their hosts. Our results show that locusts have adapted to use a pheromonal component that is derived from its digestive waste products by the action of bacteria acquired serendipitously with its food. The gut bacteria also help the locust to defend itself against microbial pathogens, mainly by producing antimicrobial phenolic compounds[8,11–13]. These contributions by the insect's gut microbiota to its behaviour and survival were previously unsuspected.

Table 1 Volatile phenolic compounds released from locust faecal pellets

Treatment	Guaiacol ($\mu g\ g^{-1}\ d^{-1}$)	Phenol ($\mu g\ g^{-1}\ d^{-1}$)
Axenic fifth instar	Not detected	0.5
Axenic mature adult	Not detected	0.3
Monoassociated fifth instar*	6.5	4.3
Monoassociated young adult	1.0	2.0
Monoassociated adult	4.9	8.7
Normal fifth instar† (wheat seedling diet)	44.5	10.7
Normal fifth instar (γ-irradiated grass diet)	4.9	13.1
Normal mature adult (wheat seedling diet)	10.6	12.3
Normal mature adult (filter paper+vanillic acid diet)	38.5	1.4
Normal mature adult (filter paper diet only)	2.6	0.7

Axenic locusts were reared according to ref. 7.
*Monoassociated insects contain the gut bacterium *P. agglomerans* and were fed a γ-irradiated diet.
†Conventional insects contain a normal gut bacterial biota. Volatiles released from locust faeces (derived from >10 insects per experiment) were analysed by gas chromatography (GC) and the identity of compounds was confirmed by GC-mass spectrometry. The amount of compound was estimated per gram of dry weight of faecal pellets. Further methodological details are available from the authors.

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Pollution

Recovery of breeding success in wild birds

We have found that the breeding success of two insectivorous forest passerines, the great tit *Parus major* and the pied flycatcher *Ficedula hypoleuca*, has markedly improved in the vicinity of a copper-smelting plant during the seven years since it reduced its emissions of heavy metals. Our results demonstrate that reduced pollution loads can positively affect breeding performance of wild bird populations over a relatively short period, even in an area that has suffered decades of heavy-metal pollution.

We collected the data around a copper smelter in Harjavalta (61° 20′ N, 22° 10′ E) in southwest Finland during 1991–97. Concentrations of heavy metals have increased in the surrounding area of the factory because of long-term deposition[1–4]. An earlier study of the same area indicated that

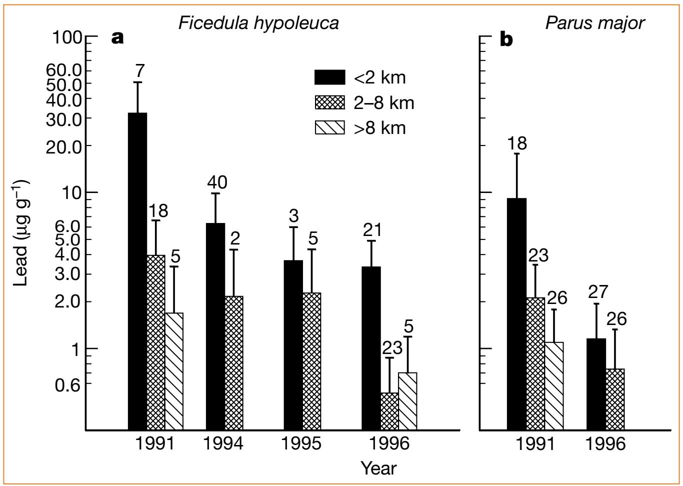

Figure 1 The median lead concentrations (µg g^{-1}, dry weight) in femurs of nestlings along the pollution gradient. **a,** *Ficedula hypoleuca*; **b,** *Parus major*. The concentration of lead was measured by atomic absorption spectrophotometry of the femur bones of 6–16-day-old nestlings found dead in their nests or dissected by us. Error bars indicate the third quartile (75%) of the data. Numbers refer to the number of individuals studied. In an ANOVA model with year and distance as independent variables, both year and zone explained significantly the femur lead concentration (log-transformed data) in both species (*F. hypoleuca*: d.f. (year) = 3, F = 17.5, P = 0.0001; d.f. (zone) = 2, F = 26.7, P = 0.0001; *P. major*: d.f. (year) = 1, F = 76.8, P = 0.0001; d.f. (zone) = 2, F = 45.7, P = 0.0001).

P. major and *F. hypoleuca* produced fewer fledglings there[3], clutch sizes of *F. hypoleuca* were small, and their eggshells were thin and porous when laid in the vicinity of the pollution source[4]. These poor-quality eggshells were linked to large amounts of heavy metals consumed by the birds in their invertebrate prey and with the paucity of calcium-rich food in the acidified forests[3,5].

Emission of sulphur dioxide decreased by about 66%, particulate dust by 63%, and lead by 95% during 1990–97. We analysed seven years' breeding results to determine whether there had been any change in breeding parameters during this period. Our analysis covered two periods, 1991–93 and 1994–97, five zones (< 1, 1–2, 2–4, 4–8 and > 8 km from the factory) and five habitat types, from the most barren to the most luxuriant, according to the ground-layer vegetation.

We found that lead concentrations in nestlings decreased in the vicinity of the factory from 1991 to 1996 by about 90% in *F. hypoleuca* and 87% in *P. major* (Fig. 1). The clutch size of *F. hypoleuca* increased significantly at points closest (< 1 km) to the reduced-pollution source, but remained the same at more distant sites (Fig. 2a). The clutch size of *P. major* failed to show a pollution-related decrease along the pollution gradient in either period, and there were no significant changes between the two periods (Fig. 2c).

The number of fledglings increased by about 1.6 chicks per nest at points close to the source in both bird species during 1991–93 and 1994–97 (Fig. 2b,d), reflecting partly the increased clutch size of *F. hypoleuca*. We found no significant effects of habitat on clutch size or on fledgling number in either species, so any changes in habitat type along the gradient do not bias the results. The relative fledgling production (fledglings in the furthest zone/fledglings in the nearest zone) correlated negatively and significantly with yearly emissions of lead (log 1,000 kg yr^{-1}) in *F. hypoleuca* ($r_s = -0.83$, $P = 0.042$, $n = 6$ years; we omitted 1994 because of insufficient data) and was marginally significant in *P. major* ($r_s = -0.71$, $P = 0.071$, $n = 7$ years).

Both bird species probably benefited from the recovery of forest vegetation. The decreased concentration of sulphuric oxides and heavy metals in tree leaves and needles probably promotes the recovery of herbivorous insect populations at the polluted sites[6,7]. This is an important change for foliage-gleaning bird species that rely strongly on tree-living insect larvae for feeding their nestlings[8].

Our results indicate that bird populations can recover relatively rapidly in an area that has suffered long-term pollution from heavy metals, as evidenced by the larger clutches of *F. hypoleuca* and the increase in fledgling production for both bird species by more than one chick during the seven-year period. The heavy-metal residues found in birds, as well as their breeding parameters, seem to reflect the level of current emissions[9] more closely than heavy-metal accumulation in the soil over the past decades.

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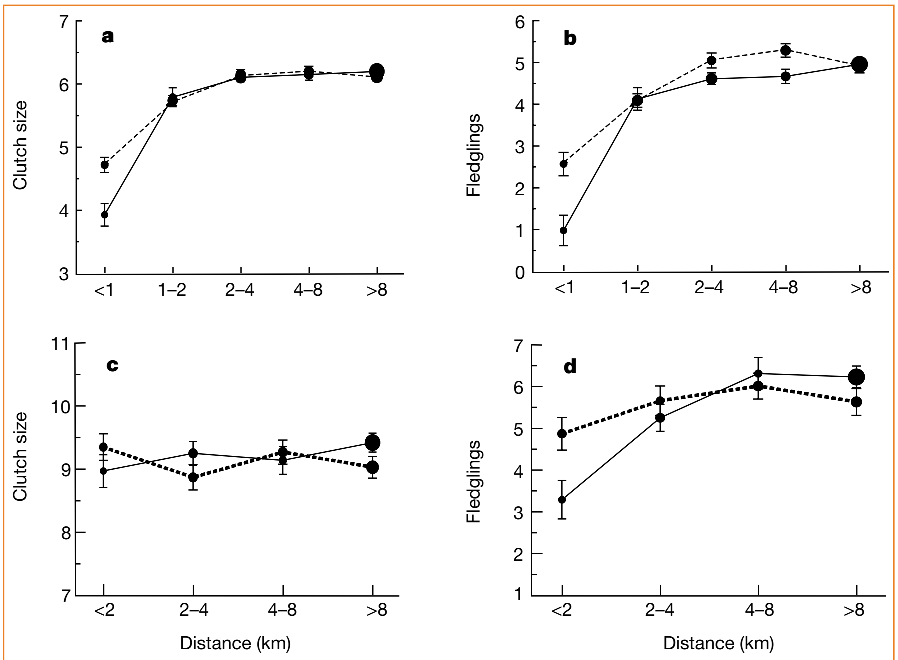

Figure 2 Clutch sizes and fledgling numbers at different distances from the pollution source. Least-square means (± s.e.) for the **a,** clutch size and **b,** fledgling number of *Ficedula hypoleuca* (*n* represents 1,562 clutches and 1,380 broods, respectively); and **c,** clutch size and **d,** fledgling number of *Parus major* (*n* represents 651 clutches and 573 broods) at five zones around the pollution source in 1991–93 (full line), and in 1994–97 (dashed line). Symbol size denotes the proportional number of nests in each zone. For *P. major*, distance zones I and II were combined in the analysis because of the small number of nests in zone I. In ANOVA models in which the time period, distance and habitat type were independent variables, the temporal change near the pollution source (time × zone) was significant for the clutch size and fledgling number of *F. hypoleuca* (d.f. = 4, F = 3.9, P = 0.0041; d.f. = 4, F = 3.9, P = 0.0036, respectively) and for fledgling number of *P. major* (d.f. = 4, F = 4.5, P = 0.0041), but non-significant for the clutch size of *P. major* (d.f. = 4, F = 2.2, P = 0.091).

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