nization. This perhaps explains why in the smaller and culturally more homogeneous eastern European communities, synchronized clapping is a daily event, whereas it happens only sporadically in western European and North American audiences. In general, our results offer evidence of a novel route to synchronization, not yet observed in physical or biological systems[2,3,5,6].

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Hormones

Leptin and diabetes in lipoatrophic mice

Lipoatrophic (lipodystrophic) diabetes is a disorder in which insulin resistance and hyperglycaemia are associated with a reduced body-fat mass[1], in contrast to the usual association of diabetes with obesity. Transgenic mice with differing degrees of fat loss can be used as models for lipoatrophy[2–4]. Using the aP2-SREBP-1c mouse[3], which has a moderate fat deficiency, Shimomura *et al.* showed that leptin treatment reverses the diabetes, concluding that insulin resistance in congenital generalized lipodystrophy can be explained by a leptin deficiency[5]. However, we have used a more severe model of lipoatrophy, the A-ZIP/F-1 mouse[2,6], in which we find that leptin treatment is only slightly effective in correcting diabetes.

A-ZIP/F-1 mice (Table 1) have an almost complete lack of white adipose (fat) tissue, a severe resistance to insulin, diabetes, and greatly reduced serum leptin levels[2]. We found that infusing leptin into A-ZIP/F-1 mice at the same rate (5 μg day^{-1} for 4 weeks, starting at 7 weeks of age) and to produce the same serum leptin level (3 ng ml^{-1}) as in Shimomura *et al.*'s mice had no effect on serum glucose or insulin concentrations (results not shown). A higher leptin dose (30 μg per day, causing leptin to rise by 5 ng ml^{-1}) did reduce glucose and insulin levels (Fig. 1), food intake (from 6.6 ± 0.3 to 4.7 ± 0.2 g day^{-1}, $P < 0.001$), and liver weight (from 3.04 ± 0.04 to 2.09 ± 0.11 g, $P < 0.001$). Even at this higher dose, however, our mice still had markedly raised blood glucose and insulin levels.

In our A-ZIP/F-1 mice, the efficacy of leptin treatment diminished with age: at 13 weeks (the age of Shimomura *et al.*'s mice), leptin had a minimal effect (Fig. 1), and no effect at all at 28 weeks (results not shown). In contrast, leptin infusion into 13-week-old leptin-deficient *ob/ob* mice completely normalized both glucose and insulin levels (Fig. 1).

Evidence from humans and mice supports the conclusion that leptin deficiency cannot completely explain the diabetic phenotype of generalized lipoatrophy. Patients with generalized lipoatrophy are more prone to diabetes[1] than are those who lack leptin[7–9]; similarly, A-ZIP/F-1 mice are more diabetic than *ob/ob* mice (Fig. 1). Thus leptin deficiency contributes to the insulin resistance of generalized lipoatrophy, but is neither the sole nor the principal cause of insulin resistance in severe forms of this disease.

The observed differences between the A-ZIP/F-1 and aP2-SREBP-1c mice are probably due to their different amounts of fat, although transgene-specific effects or their different genetic backgrounds may play a part. aP2-SREBP-1c mice have more residual adipose tissue: in these mice, leptin appears to be limiting and its replacement reverses their diabetes. A-ZIP/F-1 mice must experience loss of other functions provided by adipose tissue besides leptin secretion — for example, functions that affect fatty-acid and triglyceride metabolism. Alternatively, adipose tissue might exert a direct or indirect endocrine effect.

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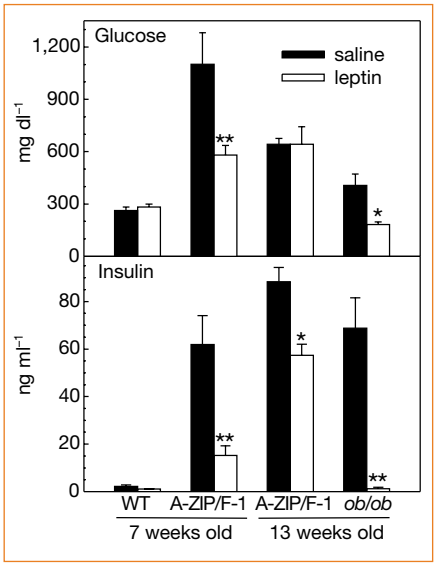

Figure 1 Effect of continuous subcutaneous leptin infusion on serum glucose and insulin concentrations. Leptin (R&D Systems) was infused into male mice by using an osmotic pump (Alzet) at 30 μg day^{-1} for six days, ending at 7 or 13 weeks of age, as indicated. Data are mean and s.e.m. with $n = 6$/group; single and double asterisks indicate differences between treated and control at $P \leq 0.01$ and $P < 0.001$, respectively. Non-fasting glucose was measured using a Glucometer Elite (Bayer) and insulin was quantified by radioimmunoassay (Linco).

Table 1 Differences in the severity of the A-ZIP/F-1 and aP2-SREBP-1c phenotypes

	Epididymal fat mass (% of wild type)	*Brown fat mass (% of wild type)*	*Glucose (mg dl^{-1}, non-fasting)*	*Insulin (ng ml^{-1}, non-fasting)*
A-ZIP/F-1	≤1	~50	~1,000	~60
aP2-SREBP-1c, line A (ref. 3)	~30	~400	~300	~20

Data are for 7-week-old mice.

Shimomura et al. *reply* — Human lipodystrophy (also called lipoatrophic diabetes) is genetically heterogeneous, with the severity of insulin resistance and diabetes mellitus varying widely depending on the degree of reduction in adipose tissue mass and the age of the patient[1,2]. It is therefore not surprising that two mouse models of lipodystrophy (created by using two different transgenes, *A-ZIP/F-1* and *aP2-SREBP-1c*) vary in their disease severity and in their sensitivity to leptin. The aP2-SREBP-1c animals respond to leptin with a decrease in their insulin and blood sugar levels[3], whereas the A-ZIP/F-1 animals of Gavrilova *et al.* apparently manifest leptin resistance. The differences between these two models should not preclude a clinical trial of leptin in leptin-deficient patients with lipodystrophy, with continuation of therapy in those who are leptin-sensitive.

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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