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Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands

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domain substituted by the hydrophobic tail of H-Ras was not (Fig. 4D). Rac1 is of particular importance to Fc receptor-mediated phagocytosis and accumulates at the base of forming phagosomes, detaching rapidly upon sealing (Fig. 4, E and F, and fig. S7A) (*10*). Rac1(Q61L) also detached from sealing phagosomes with kinetics indistinguishable from those of wild-type Rac1 (Fig. 4, G and H, and movie S7). Because Rac1(Q61L) is constitutively bound to guanosine triphosphate (GTP), its dissociation from phagosomes was not due to nucleotide hydrolysis or cessation of nucleotide exchange. Instead, release was likely mediated by termination of its electrostatic association with the plasmalemma. Accordingly, the C-terminal tail of Rac1 containing the polybasic domain behaved similarly (fig. S7B).

Our data indicate that the surface potential of the inner leaflet of the membrane decreases locally during phagosome formation. The change is attributable primarily to depletion of PIP_2 and PS, but depletion of phosphatidylinositol 4-phosphate was also observed (fig. S3 and movie S5). Activation of inositide lipases, kinases, and phosphatases occurs during phagocytosis and bacterial invasion (*3*), readily accounting for the changes in PIP_2. PS could be converted to PE by decarboxylation or could be externalized during phagocytosis by scramblases and/or efflux pumps.

Our results also indicate that the anchorage of important signaling molecules, including K-Ras and Rac1, can be modulated focally by localized changes in surface potential. Other proteins anchored electrostatically to the membrane, such as MARCKS, are equally susceptible to the charge alterations that accompany lipid remodeling. Indeed, we also obtained evidence for localized detachment of the tyrosine kinase c-Src (fig. S5, B and C).

The consequences of altered surface charge in other important biological phenomena must be considered. Activation of phosphoinositide metabolism, elevation in cytosolic calcium, and PS flipping occur after stimulation of multiple receptors and channels as well as during apoptosis. The effect of such responses on inner surface potential may be measurable with the use of approaches like the one described here. Cycles of membrane dissociation/reassociation may add a layer of functional control to complement the traditional biochemical mode of regulation of signaling proteins.

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Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands

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Despite widespread concern about declines in pollination services, little is known about the patterns of change in most pollinator assemblages. By studying bee and hoverfly assemblages in Britain and the Netherlands, we found evidence of declines (pre- versus post-1980) in local bee diversity in both countries; however, divergent trends were observed in hoverflies. Depending on the assemblage and location, pollinator declines were most frequent in habitat and flower specialists, in univoltine species, and/or in nonmigrants. In conjunction with this evidence, outcrossing plant species that are reliant on the declining pollinators have themselves declined relative to other plant species. Taken together, these findings strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species.

Anthropogenic changes in habitats and climates have resulted in substantial reductions in biodiversity among many vertebrate taxa (*1*), and evidence has been accumulating that insect biodiversity is at risk as well (*2*). Of particular concern is the possibility of community-level cascades of decline and extinction (*3*), whereby decline of some elements of the biota lead to the subsequent loss of other species that directly or indirectly rely upon them. Here we examine sets of pollinators and the plants that they pollinate to test (i) whether species that are linked to one another within communities show coincident declines and (ii) whether species with more links within communities are more robust to change because of the availability of alternative links, if an interacting species is lost.

Any loss in biodiversity is a matter of public concern, but losses of pollinating insects may be particularly troubling because of the potential effects on plant reproduction. Many agricultural crops and natural plant populations are dependent on pollination and often on the services provided by wild, unmanaged, pollinator communities. Substantial concerns have been raised about the decline or loss of these services [(*4*) but see (*5*)], culminating in formal recognition within the Convention on Biological Diversity (*6*) in the São Paulo Declaration (*7*) and the International Initiative for the Conservation and Sustainable Use of Pollinators (*8*). However, the evidence for such declines remains scanty (*5*).

To adequately demonstrate a decline in pollinator services, one would need to document (i) overall declines in pollinator density; and/or (ii) reductions in species diversity or substantial shifts in the species composition of pollinator communities, combined with changes in the distribution of traits represented in those communities (thus indicating that the loss of some pollinators has not been compensated by the rise of functionally equivalent species); and (iii) declines in either the reproductive success or abundance of plant species dependent on these pollinators. No suitable data are available to address overall pollinator density, but here we provide evidence for the remaining points, using data for bees, hoverflies, and plants from Britain and the Netherlands.

We compiled almost 1 million records for bee (all native species except the largely

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domesticated honeybee *Apis mellifera*) and hoverfly observations for both countries from national entomological databases (*9*), focusing on areas with extensive sets of observations before and after 1980. We then applied rarefaction methods to compare species richness of focal areas over each period (*10*). This approach allows valid comparisons between time periods, despite unequal sample sizes and the incorporation of records collected by many recorders who used different collecting techniques over long time spans (*10*).

Bee diversity declined in large fractions of the 10 km by 10 km cells analyzed in both countries (Fig. 1). Bee richness was measured as the number of distinct species; significant decreases in richness were observed in 52% and ~67% of British and Dutch cells, respectively, as compared with richness increases in 10% and 4% of cells in the two countries (table S1). Shifts in hoverfly diversity were less consistent (Fig. 1), with no significant directional change in richness for the UK (increases in 25% and decreases in 33% of British cells); however, increases in hoverfly richness were reported in 34%, versus decreases in 17%, of Dutch cells (table S1).

These shifts in species richness reflect shifts in the distributions of many species in both groups. Our data set does not allow direct measurement of population densities of the species involved; nonetheless, shifts over time in the relative number of records for different species can be used as an indicator of their relative frequency and ubiquity (*10*). There has been an increase in the domination of the pollinator communities of both countries by a smaller number of species. For both taxa in both countries, about 30% fewer species account for half of the post-1980 records (percentages of fewer species: British bees, 29%; British hoverflies, 29%; Netherlands bees, 32%; Netherlands hoverflies, 36%). In Britain, the species that increased were disproportionately the ones that were already common before 1980; however, in the Netherlands, this was not the case (*11*).

The functional diversity of pollination networks contributes to the maintenance of diversity in plant communities (*12*), with different groups of pollinators being complementary in their pollination services and different groups of plants being complementary in their roles as food plants for pollinators. Consequently, a decline in pollinator diversity might have little effect on a community if the fluctuating species were functionally similar. However, the traits of increasing and declining species of solitary bees and hoverflies differ in consistent ways (Table 1). In both countries and in both groups, species with narrow habitat requirements have experienced greater relative declines. In solitary bees, oligolectic species (those using few flower taxa as food sources) have declined significantly in Britain, and long-tongued taxa have declined significantly in the Netherlands. Dietary specialization is important in hoverflies as well, with both adult and larval diets being strongly related to changes in hoverfly occurrence. Migratory hoverflies have fared better than nonmigratory species in both countries. In Britain, bee and hoverfly declines are greater among species with only a single generation per year; however, this pattern is not found in the Netherlands. The significant trends indicate that specialized species [i.e., in habitat and dietary requirements and, arguably, tongue length (*12*, *13*)] and species characterized by slower development and lower mobility (those having fewer generations per year and being nonmigratory) tend to decline more than generalist, fast developing, and more mobile species.

Such shifts in pollinator traits suggest possible shifts in pollination services. Indeed, recent experiments have shown that the functional diversity of pollinators can affect diversity in plant communities (*12*). We know of no data that will allow us to assess directly whether rates of pollinator visitation or pollen deposition to flowers have shifted appreciably in Britain or the Netherlands. We can, however, examine shifts in plant species distributions using floral inventories from both countries (*10*, *14*, *15*) to see whether shifts in plants are consistent with the observed shifts in pollinators. In Britain, obligately outcrossing plants reliant on insect pollinators were declining on average; species reliant on abiotic (wind or water mediated) pollination were increasing; and self-pollinating plant species showed an intermediate response (Table 2). In the Netherlands, changes were not significantly different among these three groups; however, given the observed decline in bees and increase in hoverflies in the Netherlands, divergent trends between bee-pollinated plants and other insect-pollinated plants may be expected there. After reexamining the data on the insect taxa reported as pollinators of outcrossing plants (*15*), we found that, on average in the Netherlands, plants that were exclusively pollinated by bees were declining, but plants pollinated by flies and other insects (including bees) were increasing. If the changes among bee-pollinated outcrossers, out-

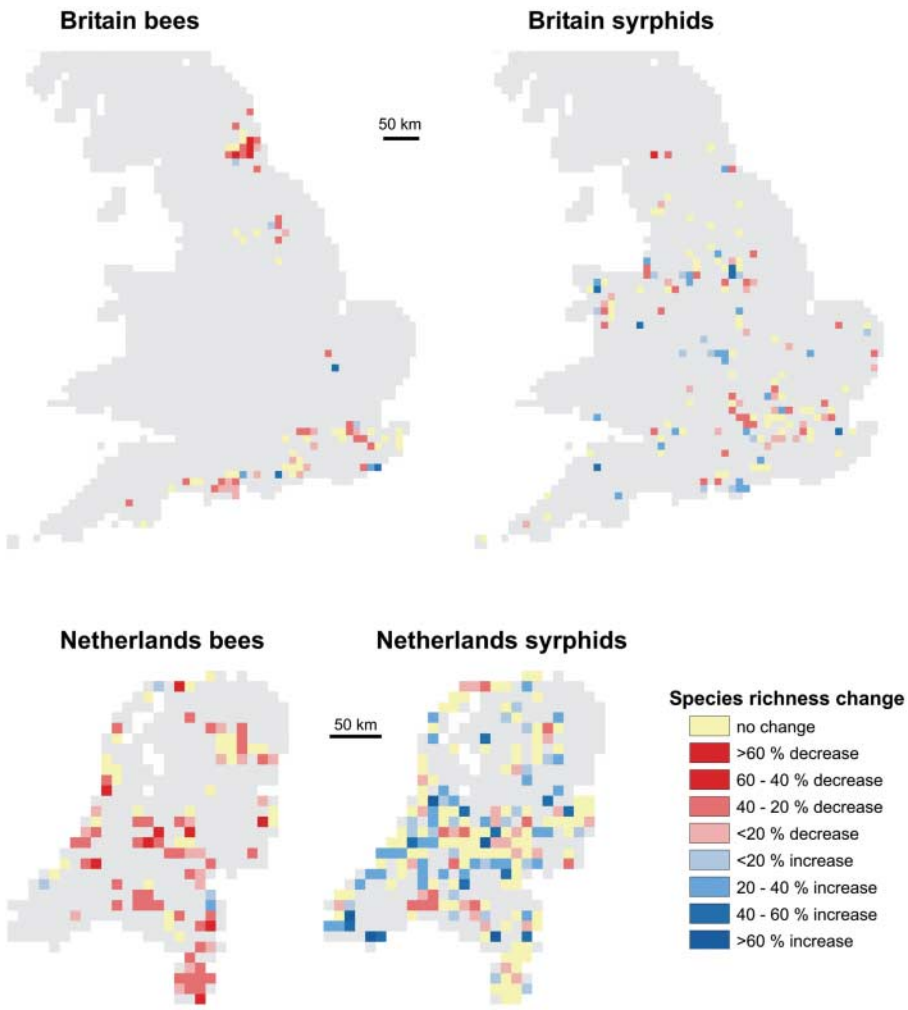

Fig. 1. Bee and hoverfly richness has changed in many of the 10 km by 10 km cells analyzed for Britain and the Netherlands. Some British cells contained adequate data only on eusocial or only on solitary bees (*10*). Changes in species richness were calculated from rarefaction analyses (*10*).

crossers with abiotic pollination, and predominantly self-pollinating plants are compared, the trends observed in the Netherlands mirror those for Britain: Bee-dependent plants have declined, abiotically pollinated plants have increased, and plants mainly relying on self-pollination have shown an intermediate response (Table 2).

We cannot tell from these data whether the decline of the plants precedes the loss of the associated pollinators, whether the decline of the pollinators leads to the loss of reproductive function and then to the decline of the plants, or indeed whether the plants and their pollinators are both responding to some other factor. However, the results clearly show that linked elements in biological communities (i.e., specialist pollinators and the obligately outcrossed plants that they pollinate) are declining in tandem. Furthermore, the difference between the two countries implies that there is probably a causal link, because it is the corresponding groups of plants and pollinators in both countries that are changing. The hypothesis that species that rely on a broader range of other species within a community are more robust in the face of change is supported by the following evidence: Pollinators that rely on few plants for their resources have declined the most, whereas generalists have prospered [compare with (*16*)]. Moreover, the decline of bees (specialized as pollinators) relative to hoverflies (having broader feeding habits) could be interpreted in this light.

Demonstrating that there are shifts in pollinator assemblages and associated changes in wild plant communities in two countries does not prove the existence of a global pollination crisis. Britain and the Netherlands are not only two of the countries with the best available data but also two of the most densely populated and anthropogenically modified landscapes on the planet. Few British habitats can be thought of as truly natural, and in the Netherlands the landscape is largely artificial. Nonetheless, it seems probable that shifts similar to those documented for these countries will be found in other parts of northwest Europe and, increasingly, in other regions (*17*). Documenting the geographical extent of the declines shown here is a priority for future research. It is also important to begin mechanistic studies of the causes of these declines, with habitat alteration (*18*), climate change (*19*–*21*), and agricultural chemical usage (*18*, *22*) being potential key drivers of observed shifts (*23*).

Table 1. Trait-based patterns in pollinator declines. Proportions are based on species that showed significant change in the number of cells (*n*) in which they were reported during the two time periods (pre- and post-1980). Declining solitary bee and hoverfly species tend to be found more among the specialists (characterized by narrow habitat ranges, limited dietary choice, slower development, and greater residency) than among generalist species (characterized by wide habitat ranges, broader dietary choice, multiple generations per year, and greater tendency toward migration). Traits were assigned by using methodologies in (*25*) and (*26*). Bumblebees and honeybees were excluded from the analysis (*10*). Oligo, oligolectic; Poly, polylectic; Uni, univoltine; Multi, multivoltine; Macro, macroorganisms; Micro, microorganisms; Res, resident; Mig, migrant.

	Britain				Netherlands			
	Solitary bees							
Trait	Trait category (proportion declining)		*P*	*n*	Trait category (proportion declining)		*P*	*n*
Habitat range	Narrow (0.90)	Wide (0.25)	0.0001	32	Narrow (0.83)	Wide (0.53)	0.090	29
Flower specificity	Oligo (0.86)	Poly (0.41)	0.034	34	Oligo (0.55)	Poly (0.76)	0.198	36
Tongue length	Long (0.70)	Short (0.41)	0.099	56	Long (1.00)	Short (0.51)	0.028	49
Generations	Uni (0.60)	Multi (0.14)	0.042	44	Uni (0.76)	Multi (0.55)	0.433	42
	Hoverflies							
Trait	Trait category (proportion declining)		*P*	*n*	Trait category (proportion declining)		*P*	*n*
Habitat range	Narrow (0.96)	Wide (0.28)	0.0001	53	Narrow (0.52)	Wide (0.25)	0.025	67
Adult food	Narrow (0.63)	Wide (0.41)	0.095	60	Narrow (0.53)	Wide (0.16)	0.0001	86
Larval food	Macro (0.74)	Micro (0.43)	0.009	59	Macro (0.59)	Micro (0.20)	0.002	79
Generations	Uni (0.80)	Multi (0.29)	0.0001	50	Uni (0.43)	Multi (0.38)	0.63	88
Migration	Res (0.63)	Mig (0.20)	0.01	64	Res (0.46)	Mig (0.17)	0.025	88

Table 2. Mean relative change (±SE) in distribution of British (*27*) and Netherlands (*28*) plant species according to their main pollen vector (*10*). Insect-pollinated outcrossing plants in Britain and bee-pollinated outcrossing plants in the Netherlands have declined, whereas plants with abiotic pollination have increased. Plant breeding systems were derived by combining the ECOFLOR (*29*) and BIOLFLOR (*30*) databases (*10*). British data were tested with an analysis of variance and a post hoc Tukey test. Netherlands data were tested with a Kruskal-Wallis test and a post hoc multiple comparison test. Superscripts indicate group differences based on post hoc tests. *n*, number of plant species; NL, Netherlands.

	Obligatory outcrossing, insect pollinated	Obligatory outcrossing, wind or water pollinated	Predominantly self pollinating	*P*
Britain	−0.22 ± 0.06* (*n* = 75)	+0.18 ± 0.14† (*n* = 30)	−0.003 ± 0.70*† (*n* = 116)	0.009
Netherlands	+0.10 ± 0.08 (*n* = 182)	+0.18 ± 0.08 (*n* = 160)	−0.08 ± 0.11 (*n* = 143)	0.091
NL bee plants	−0.12 ± 0.13* (*n* = 42)	+0.18 ± 0.08† (*n* = 160)	−0.08 ± 0.11*† (*n* = 143)	0.036

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Supporting Online Material

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Crystal Structure of a Divalent Metal Ion Transporter CorA at 2.9 Angstrom Resolution

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CorA family members are ubiquitously distributed transporters of divalent metal cations and are considered to be the primary Mg^{2+} transporter of Bacteria and Archaea. We have determined a 2.9 angstrom resolution structure of CorA from *Thermotoga maritima* that reveals a pentameric cone–shaped protein. Two potential regulatory metal binding sites are found in the N-terminal domain that bind both Mg^{2+} and Co^{2+}. The structure of CorA supports an efflux system involving dehydration and rehydration of divalent metal ions potentially mediated by a ring of conserved aspartate residues at the cytoplasmic entrance and a carbonyl funnel at the periplasmic side of the pore.

Divalent metal cations are essential cofactors in many proteins. To provide cells with appropriate concentrations of divalent metal cations, highly regulated transporters and channels have evolved to translocate these ions across the hydrophobic membranes. CorA is one of the best studied families of divalent cation transporters (*1*–*9*). It is considered to be the primary Mg^{2+} transporter of both Bacteria and Archaea and is ubiquitously distributed (*8*). Sequence homologies between members of this family are most pronounced at the C termini; sequence conservation in the N termini is less significant (fig. S1). The overall sequence similarity between eukaryotes and prokaryotes is weak, except for the highly conserved Gly-Met-Asn (GMN) motif close to the C termini (*10*, *11*). Nevertheless, some eukaryotic CorA family members show overlapping activities with the prokaryotic members that suggests functional, as well as structural, conservation (*4*, *10*, *12*, *13*). Studies of CorA from *Salmonella typhimurium*, *Escherichia coli*, and the Archaeon *Methanococcus jannaschii* demonstrate that ions can be transported in both directions (*1*–*3*, *5*, *8*, *9*).

Kehres and Maguire recently reported two classes of CorAs among Bacteria and Archaea (*8*). The second class, CorA-II, which differs from the extensively studied *S. typhimurium* and *E. coli* CorAs, was suggested to contain two transmembrane helices, with both termini in the cytosol. In the same report, the CorA-II proteins were suggested to be efflux systems. Moreover, a novel CorA-related protein, ZntB, was recently identified in *S. typhimurium* (*14*). ZntB was shown to be a Zn^{2+} efflux system with two predicted transmembrane helices and both termini facing the cytosol (*15*). This is in contrast with the predicted topology of *S. typhimurium* and *E. coli* CorA, with three transmembrane helices and the N terminus facing the periplasm.

Here, we report the crystal structure of a full-length CorA homolog from *Thermotoga maritima*, at 2.9 Å resolution. The recently reported structure of a pentameric full-length CorA at 3.9 Å was used for molecular replacement, revealing two pentamers in the asymmetric unit (*16*). The structure has been refined to an R of 27.6% and an R_{free} of 29.5% with good stereochemistry (table S1).

The CorA structure reveals a pentamer with the shape of a cone (Fig. 1). The tip of the cone is formed by two transmembrane (TM) helical segments from each monomer and the large opening of the cone by the N-terminal region of CorA. The fold of the CorA monomer is composed of an N-terminal α/β domain with a central seven-stranded mixed β sheet lined by three small helices. Two long α helices cover one face of the α/β domain and form a bundle together with a giant α helix 7 constituted by ~70 residues. The C-terminal end of helix 7 constitutes the first transmembrane segments (residues 291 to 312). Following the large helix 7 is helix 8 (residues 327 to 349), which forms the second TM helix and packs in a ring around the TM segment of helix 7 (Fig. 1).

Thermotoga maritima CorA is most closely related to the class II CorA with both N- and C-terminal ends facing the cytosol and, therefore, is likely to be primarily involved in ion efflux. The localization of the N termini in the cytoplasm is also supported by the positive-inside rule (*17*) and the N_{in}-C_{in} topology of "helical hairpin" structures (*18*). Sequence alignment of close homologs of *T. maritima* CorA and those of *S. typhimurium* CorA support the proposal for two distinct classes of CorA (fig. S1).

Our structure agrees in all general features with the structure determined by Lunin *et al.* (*16*) that was used for phasing. However, because of the higher resolution of 2.9 Å, our structure provides more details of functionally important regions, including potential regulatory metal binding sites beyond the metal in site 1 (M1) identified in the 3.9 Å structure. Two putative metal-binding sites are found at each interface between the N-terminal domains in the pentamer (Fig. 2). An anomalous difference map of Co^{2+}-soaked crystals shows that Co^{2+}

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