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IDEA AND PERSPECTIVE

Ecological interactions drive evolutionary loss of traits

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Abstract

Loss of traits can dramatically alter the fate of species. Evidence is rapidly accumulating that the prevalence of trait loss is grossly underestimated. New findings demonstrate that traits can be lost without affecting the external phenotype, provided the lost function is compensated for by species interactions. This is important because trait loss can tighten the ecological relationship between partners, affecting the maintenance of species interactions. Here, we develop a new perspective on so-called 'compensated trait loss' and how this type of trait loss may affect the evolutionary dynamics between interacting organisms. We argue that: (1) the frequency of compensated trait loss is currently underestimated because it can go unnoticed as long as ecological interactions are maintained; (2) by analysing known cases of trait loss, specific factors promoting compensated trait loss can be identified and (3) genomic sequencing is a key way forwards in detecting compensated trait loss. We present a comprehensive literature survey showing that compensated trait loss is taxonomically widespread, can involve essential traits, and often occurs as replicated evolutionary events. Despite its hidden nature, compensated trait loss is important in directing evolutionary dynamics of ecological relationships and has the potential to change facultative ecological interactions into obligatory ones.

Keywords

Comparative genomics, compensated trait loss, dietary composition, gene loss, mutualism, parasitism, symbiosis.

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INTRODUCTION

A new perspective on trait loss

A key goal in evolutionary biology is to unravel why novel characters arise and how these characters contribute to an individual's fitness. In contrast, much less importance has been attributed to the evolutionary loss of characters. Evolutionary loss of traits can occur if traits are selected against or if a trait becomes redundant. This is typically driven by a weakening or removal of the selection pressure maintaining the trait (Maughan *et al.* 2006; Lahti *et al.* 2009). Over the last several decades there has been an increased appreciation of trait loss processes (Lehmann *et al.* 2007), now leading to questions about the role of ecological context in trait loss dynamics. Traits tend to have a higher chance of being lost if there is relaxed selection (Lahti *et al.* 2009), but what specific ecological conditions drive relaxed selection?

This question is particularly hard to answer because it is often difficult to detect incipient loss of characters in the field. Under natural conditions, trait loss is usually only detected when the phenotype is severely affected. However, at more advanced stages of degradation, there can be difficulties in deducing the ecological conditions present when selection was first relaxed. The initial stages of trait loss are rarely recognised, partly because of the more subtle effects of early decay on trait function (Tobler *et al.* 2010). Similar to concepts of evolutionary acquisition and adaptation of traits, trait loss can occur in various gradations, ranging from complete loss of a trait to vestigialisation, when the trait is still present in a degraded form. Because trait loss can be a gradual process, initial stages of regression may be indistinguishable from naturally occurring individual variation in trait values. To predict newly developing trait loss, one would need to identify the direction and magnitude of any remaining selection pressures on the trait, which is notoriously difficult (Lehmann *et al.* 2007).

The majority of trait loss research has focused on cases in which a change or shift in the environment has driven functional redundancy. However, trait loss can also occur despite a continued functional requirement of the phenotype. In this type of trait loss, the phenotypic function of the trait persists because the organism has evolved some sort of dependency on a biological partner to provide this function. Here we coin the term 'compensated trait loss' to describe trait loss without loss of function due to provision of resources by ecological interactions (Visser *et al.* 2010). Because we do not necessarily see radical changes in phenotypic function, compensated trait loss has remained largely unstudied and its occurrence is likely to be grossly underestimated. Although earlier work on trait loss in endosymbionts and parasites have recognised this phenomenon (Dedeine *et al.* 2001; Dale & Moran 2006), a systematic study of its scope, frequency and implications has been lacking so far.

A renewed research focus on evolutionary loss of traits is needed to create a framework for how and why compensated trait loss occurs. Recent technological advances now allow us to detect compensated trait loss not only through ecological experiments but also by searching the entire genome for gene degradation and deficiency of orthologous genes (Wyder *et al.* 2007; Kirkness *et al.* 2010; Werren *et al.* 2010; Zhang *et al.* 2010). Quantifying its true extent is important because compensated trait loss may help explain the long-term stability of co-evolved interactions such as mutualism and parasitism.

Here, we develop a new perspective on trait loss and the evolutionary dynamics between interacting organisms. We will: (1) present the concept of compensated trait loss and discuss its ecological consequences; (2) compile the known cases of compensated trait loss

to identify which factors promote compensated trait loss and (3) present prospects for studying compensated trait loss, including the use of genomic information.

COMPENSATED TRAIT LOSS THROUGH ECOLOGICAL INTERACTIONS

In this section, we formulate the concept of compensated trait loss into an explicit hypothesis and discuss specific features that follow from the current evidence for compensated trait loss.

The two defining features of compensated trait loss are: (1) the actual loss of a trait and (2) the supply of a function or resource by an ecological partner. For clarity, Table 1 contains key definitions of terms we will apply throughout the text. We define trait loss as *a genetic change leading to the absence of the trait phenotype under environmental conditions that induced trait expression in the ancestral lineage*. In compensated trait loss, a trait is prone to loss because its function is provided by an ecological partner and this eliminates positive selection for the underlying trait. As a consequence, compensated trait loss leads to functional dependence on an ecological partner (Dale & Moran 2006; Wu *et al.* 2006; Xu *et al.* 2007; Timmermans & Ellers 2009; Suen *et al.* 2011). Functional dependency has been considered previously in the evolutionary literature (Table 1). A related concept is evolved dependence, in which a mutualistic species has lost its ability to perform well without their partner (Douglas and Smith 1989; de Mazancourt *et al.* 2005). However, a key difference is that evolved dependence concerns all benefits from mutualisms, many of which do not involve trait loss but trait acquisition. For example, fungal endophytes can increase heat resistance of grasses, allowing them to colonise otherwise inhospitable habitat; or grazing can lead to increased seed production in plants through overcompensation (de Mazancourt *et al.* 2005). In such cases, ecological interaction produces ultimate benefits, but not trait loss.

A well-known concept included in compensated trait loss is genome reduction in endosymbionts. The extreme forms of trait loss and extensive genome reduction found in bacterial symbionts is one of the most powerful illustrations of how ecological interactions can drive relaxed selection and trait loss. Bacterial symbionts can have up to five to ten times smaller genomes than free-living bacteria (McCutcheon & Moran 2012). Although a substantial part of the reduction in genome size may result from small effective population sizes in strictly host-associated symbionts, host-compensatory mechanisms certainly play an important role in the degradation of essential genes. More research is needed to establish what proportion of the reduction in genome size is a direct consequence of compensated trait loss. Furthermore, the newly presented 'Black Queen Hypothesis (BQH)' also explains endosymbiont genome size reduction through compensated trait loss (Morris *et al.* 2012), but at the

Table 1 Definitions of key terms and an overview of previous perspectives on relaxed selection and trait loss

Definition		
Trait	Any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organisation	Violle *et al.* (2007)
Phenotype	The observed expression of a specific trait for an individual, based on genetic and environmental effects	Lynch & Walsh (1998)
Function	The mode of action by which phenotypic expression of the trait is linked to fitness	Calow (1987)

Concepts	Compensation of function?	Species interaction?	Description	Refs.
Trait loss through relaxed selection	No	Possible, not necessary	Trait loss due to reduction of abiotic or biotic selection pressures. Also the function of the trait is lost, since this function is redundant in the new environment. Examples: Loss of cold resistance in warmer areas; Loss of sexual behaviour in parthenogenetic species	Lahti *et al.* (2009)
Evolved dependency	No	Yes	Loss of ability to perform well without mutualistic partner due to adaptation, which may involve trait loss or trait acquisition. Originally developed for endosymbionts, but later applied to mutualism in general. Examples: Dependence of forb flowering on grazing	Douglas and Smith (1989), Mazancourt *et al.* (2005)
Environmental compensation	Yes	No	Trait loss in which the function of the lost traits is compensated for by abiotic factors. Examples: Catalysis of vitamin D production by sunlight; Loss of calcified abdominal armour in hermit crabs, due to protection from empty shells or hollow stone	
Black queen hypothesis	Yes	Possible, not necessary	No direct interaction between species necessary, benefit is provided as a public good. Developed in the context of bacterial communities. Examples: detoxification of HOOH by some members of the marine microbial community inevitably benefits other cells in their vicinity, leading to loss of oxidative stress genes	Morris *et al.* (2012)
Genome reduction in endosymbionts	Possibly, not necessary	Yes	Large scale loss of genes in bacterial endosymbionts. Examples: Mealybug endosymbiont *Tremblaya princeps* has a genome smaller than 300 kb	McCutcheon & Moran (2012)
Compensated trait loss through species interactions	Yes	Yes	Trait loss due to provision of trait function by an ecological partner. Trait loss is hidden at the functional level as long as ecological interaction is maintained. Examples: Loss of lipogenic ability in parasitic fungus that feeds on host's skin lipids; loss of arginine biosynthesis in fungus-farming ants	Visser *et al.* (2010)

References are listed in Appendix S1.

individual level through the production and utilisation of public goods. The BQH poses that many genetic functions are leaky and unavoidably produce public goods that are available to the bacterial community. As long as some individuals continue to produce these public goods, the function is dispensable for other individuals and selection will favour its loss (Morris *et al.* 2012). No applications of this theory have been made beyond microbial communities.

Ideally, proof of compensated trait loss would include three types of evidence: (1) ecological experiments to support the notion that the lost function is provided by an ecological partner; (2) molecular work to show degradation or silencing of the genes underlying the lost trait and (3) phylogenetic analysis to demonstrate the presence of the trait in ancestral species and non-symbiotic sister species. A few cases of compensated trait loss have been studied in sufficient detail to provide all three types of evidence, for example loss of digestive organs in some marine worms that rely on chemosynthetic symbionts for nutrient uptake (Dubilier *et al.* 2008), and loss of lipogenesis in parasitoids (Visser *et al.* 2010). Several other cases of compensated trait loss are only supported by molecular evidence [e.g. loss of cysteine synthesis in *Acropora* corals (Shinzato *et al.* 2011)], or experimental manipulation [e.g. loss of oocyte production in *Wolbachia*-infected insects (Dedeine *et al.* 2001; Pannebakker *et al.* 2007)].

To quantify the current evidence for compensated trait loss and to identify trends in its occurrence, Table 2 presents an overview of cases of compensated trait loss compiled from a comprehensive literature survey across all taxonomic groups. We include cases for which at least one type of evidence is present. Because the classical cases of genomic reduction in endosymbiont bacteria have been covered previously (McCutcheon & Moran 2012), these examples are not fully represented in our table. Importantly, the examples in Table 2 may not reflect the true frequency of compensated trait loss because research efforts may be largely biased by the interest researchers have put on symbiosis in some groups. With this caveat in mind, we reached four main conclusions from our survey: (1) compensated trait loss is widespread across taxa, (2) interactions between free-living organisms can drive compensated trait loss, (3) compensated trait loss can cause organisms to lose essential traits without loss of the function provided by the trait and (4) compensated trait loss often occurs as replicated evolutionary events. We discuss these four points below.

Compensated trait loss is widespread across taxa

Compensated trait loss occurs across diverse taxonomic groups (Table 2, Fig. 1). However, the distribution of known cases is not equal over the Kingdoms; we find the majority of studies reporting compensated trait loss in fungi and invertebrates. In contrast, only a few cases have been reported in mammals and none at all in birds or fish. Moreover, the relatively small number of fully sequenced genomes of vertebrate species hinders detection of compensated trait loss in the higher taxa. Unless detailed comparative genomic information is available, as is the case for bacteria, it is highly likely that the vast majority of instances have yet to be uncovered. Fortunately, the ever-growing sequencing effort should reduce the taxonomic bias in genomic data, allowing us to test if there is a true skew in taxonomic distribution of instances of compensated trait loss.

One striking feature of compensated trait loss is that there appear to be no constraints as to which species experiences or facilitates trait loss. For example, some fungi that parasitise lipids from their vertebrate host's skin have lost the capability for lipid synthesis (Xu *et al.* 2007). Other fungi are themselves the victims of parasitism by mycoheterotroph plants that consume their carbon, allowing these plants to lose their ability to photosynthesise (Fig. 1; Motomura *et al.* 2010). In mutualistic relationships, such as between endosymbionts and their hosts, both parties show compensated trait loss. For example, *Buchnera* bacteria have undergone genome reduction as outlined above, but their aphid hosts have also lost genes. In the pea aphid *Acyrthosiphon pisum*, several genes involved in amino acid biosynthesis are lost, which are encoded in the *Buchnera* genome (Richards *et al.* 2010).

Compensated trait loss can even occur as nested symbioses in communities of symbionts and hosts. The arbuscular mycorrhizal fungus *Gigaspora margarita* – which is obligately dependent on plant hosts for carbon – is itself host to a dependent bacterial endosymbiont (Ghignone *et al.* 2012). Complex metabolic interdependence has also been documented among endosymbionts within several individual insect hosts. For example, the xylem sap-feeding sharp shooters have two bacterial symbionts with striking complementarity in the remaining metabolic capabilities: *Sulcia muelleri* is responsible for essential amino acid biosynthesis, while *Baumannia cicadellinicola* contributes vitamins and cofactors within this tripartite symbiosis (McCutcheon & Moran 2007). These types of compensated trait losses are likely widespread in more communities (e.g. consortia of microbes within animal guts) and could also occur in other types of ecological interactions, but can be difficult to identify due to the number of interacting partners.

Interactions between free-living organisms as a driver of compensated trait loss

Although obligately host-associated organisms, such as blood parasites or endosymbiont bacteria, are particularly susceptible to compensated trait loss, trait loss is also frequently observed in free-living species. In these cases, both partners reproduce independently, and the interaction has to be established anew in each subsequent generation. These examples of compensated trait loss are more difficult to explain because the relationship between free-living interacting organisms can be (temporarily) broken up. Such intervals could reinstate selection pressure on redundant traits, thus potentially preventing trait loss.

Table 2 contains several examples of compensated trait loss resulting from interactions between free-living organisms, such as the case of frugivory which has caused primates and other mammals to lose the ability to synthesise vitamin C. The presence of excess amounts of this vitamin in the diet has rendered *de novo* synthesis redundant and mutation accumulation has been shown to compromise the gene involved in vitamin C production (Chatterjee 1973; Ohta & Nishikimi 1999). If there is a shift in diet, as for example occurred in 15th–18th century sailors that were devoid of fresh fruits and vegetables containing vitamin C, the detrimental effects of the loss of vitamin C synthesis become apparent and cause scurvy. Frugivory is therefore an obligate interaction, despite the lack of physical association between primates and fruit-bearing trees.

Ant-plant mutualisms, in which ants offer protection to plants from herbivores in return for nutritional resources from the host plant, is another example of free-living organisms experiencing compensated trait loss. In a study by Heil *et al.* (2005), the authors

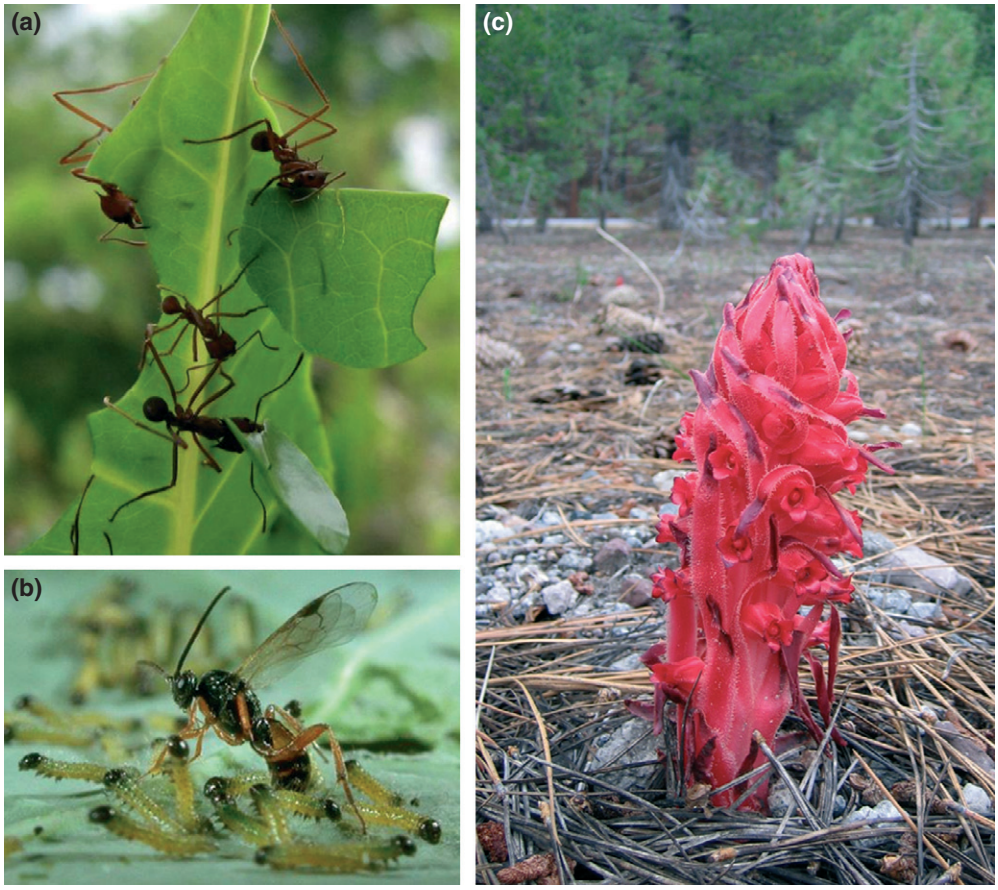

Figure 1 Functions or resources provided by ecological interactions drive compensated trait loss in partners. (a) Leaf-cutter ants (*Atta* sp.) collecting pieces of fresh plant material to manure fungal gardens. The ants' obligate dependence on the fungus for nutrients has led to reductions in genes related to nutrient acquisition (photo: Jarrod Scott). (b) A parasitoid wasp, *Cotesia glomerata*, laying eggs in the larvae of its host, *Pieris brassicae*. The majority of parasitoids species have lost the ability for lipogenesis, probably because of their ability to manipulate host lipid resources (photo: Hans Smid). (c) The inflorescence of the mycoheterotroph *Sarcodes sanguinea* (Ericaceae). Mycoheterotrophic plants derive their carbon from parasitism upon fungi, and have lost the ability for photosynthesis (photo: Vincent Merckx).

examined the relationship between the chemical composition of extrafloral nectar of *Acacia* plants and their symbiont *Pseudomyrmex* ants. The carbohydrate composition of myrmecophyte *Acacia* extrafloral nectar is distinctive because it is devoid of sucrose due to the presence of the sucrose-cleaving enzyme invertase. As a reciprocal adaptation, obligate symbiont ants were shown to lack activity of this essential enzyme in their guts. In contrast, non-symbiont ant species or facultative *Pseudomyrmex* species had normal levels of invertase activity and preferred extrafloral nectar containing sucrose. In this case, compensated trait loss potentially provides the *Acacia* plants with a protection against exploitation by non-symbiont ants.

Extreme compensated trait loss

Trait loss can be difficult to study because many lost traits are undetectable to the phenotype and go unnoticed. However, in compensated trait loss even basal traits that define entire classes of organisms have been lost. Plants are, for example, defined by their ability to capture food via photosynthesis. However, loss of photosynthetic ability (i.e. loss of functional chloroplasts and thus a definitional movement from a 'plant' to an 'animal') has occurred with surprising frequency in eukaryote evolution (Table 2, de Castro *et al.* 2009). Roughly 10% of land plants rely on a heterotrophic interaction for their survival, meaning they invade the tissue of a photosynthetic host plant for carbon (parasitism) or obtain carbon from a root-associated fungus (mycoheterotrophy), rather than solely relying on their own photosynthetic power (Westwood *et al.* 2010). Once a heterotrophic lifestyle has been adopted and selection for photosynthetic capacity is relaxed, loss of plastid gene content can be rapid; one study found that 30 or more chloroplast genes were lost within $\sim 5 \times 10^6$ years (Depamphilis & Palmer 1990). The end result is that heterotrophic plants have evolved incredibly reduced plastid genomes. The mycoheterotroph *Rhizanthella gardneri*, an underground orchid, has the smallest organelle genome ever described in land plants. At roughly 59 200 bp, it is the least gene-rich plastid genome known apart from the fragmented plastid genome found in some dinoflagellates (Delannoy *et al.* 2011).

On the other end of the compensated trait loss extreme are animals, typically characterised by mouths and guts for digesting food. Like plants, animals have also forgone these defining traits via compensated trait loss. In marine ecosystems, efficient feeding processes provided by chemosynthetic symbionts – symbionts that use a chemical compound as an energy source to synthesise organic carbon – have enabled worms to reduce their guts, mouth and anus. Hundreds of species in at least seven different phyla show loss of traits for digestive and/or excretory system (Table 2, Dubilier *et al.* 2008). The advantages of reducing the digestive system seems to

Table 2 An overview of the occurrence of compensated trait loss from a comprehensive literature survey across all taxonomic groups. Only those cases are included for which at least one type of evidence is present (P = Phylogenetic, M = Molecular, E = Ecological)

Phylum	Species	Trait lost	Resource provider	Evidence	Interaction	Description
Mammals	Primates, guinea pigs, fruit bats	Vitamin C synthesis	Fruit-bearing plants	P, M, E	Frugivory	Dietary vitamin uptake makes *de novo* synthesis of ascorbic acid redundant[1,2]
Insects	Parasitic Hymenoptera	Fatty acid synthesis	Arthropod hosts	P, M, E	Parasitism	Host exploitation of lipids renders lipid synthesis redundant[3,4]
Insects	Parasitic Diptera and Coleoptera	Fatty acid synthesis	Arthropod hosts	P, E	Parasitism	Host exploitation of lipids renders lipid synthesis redundant[3]
Insects	Aphids and Leafhoppers	Amino acids	Endosymbiont bacteria *Buchnera* and *Baumannia*	M, E	Endosymbiont	Endosymbionts provide essential amino acids[5,6,7]
Insects	Sharp shooters	Amino acids; vitamins and co-factors	Endosymbiont bacteria *Baumannia* and *Sulcia*	M	Endosymbiont	Endosymbionts provide either amino acids, vitamins or co-factors to host[8]
Insects	*Teleogryllus oceanicus*	Calling behaviour, reduced stridulation structures	Conspecific calling morphs	E	Parasitism	Conspecifics are exploited for their calling behaviour[9]
Insects	*Asobara tabida*	Regulation of apoptosis	Endosymbiont bacteria: *Wolbachia*	E	Endosymbiont	Presence of *Wolbachia* is necessary for host oogenesis[10,11]
Insects	Tsetse fly	Vitamin B synthesis	Endosymbiont bacteria: *Wigglesworthia*	M, E	Endosymbiont	Vitamin B synthesis by endosymbiont makes *de novo* synthesis redundant[12]
Insects	*Acromyrmex echinatior*	Arginine synthesis, detoxification genes	Fungi	M	Mutualism	Symbiotic fungus synthesises arginine and provide food for ants[13]
Insects	*Atta cephalotes*	Serine proteases, arginine biosynthesis and hexamerin	Fungi	M	Mutualism	Symbiotic fungus synthesises serine proteases, arginin and hexamerin[14]
Insects	*Pseudomyrmex* species	Sucrose-cleaving enzyme invertase	Myrmecophyte plant: *Acacia*	P, E	Mutualism	Extrafloral nectar of *Acacia* contains invertase activity[15,16]
Insects	Cynipid wasp inquilines	Gall induction ability	Cynipid gall inducers	P, E	Parasitism	Exploitation of cynipid galls caused secondary loss of gall induction ability[17]
Insects	Cockroaches	Nitrogen assimilation	*Blattabacterium*	M	Endosymbiont	Bacteria recycle nitrogen from urea and ammonia into glutamate for host use[18]
Collembola	*Folsomia candida*	Egg viability	Endosymbiont bacteria: *Wolbachia*	E	Endosymbiont/ Parasitism	Presence of *Wolbachia* is necessary for egg development[19]
Worms	*Olavius algarvensis*	Digestive and excretory systems	Extracellular chemo-synthetic endosymbionts	P, M	Endosymbiont	Endosymbionts process and provide nutrients[20]
Worms	*Riftia pachyptila*	Digestive tract	Extracellular chemo-synthetic endosymbionts	P, M	Endosymbiont	Endosymbionts process and provide nutrients[20,21]
Worms	*Astomonema* sp.	Mouth	Chemosynthetic symbionts	P, M	Endosymbiont	Endosymbionts process and provide nutrients[20,22]
Worms	*Osedax* sp.	Mouth, stomach and gut	Heterotrophic symbionts	P, M	Endosymbiont	Endosymbionts process and provide nutrients[20]
Worms	*Schistosoma mansoni*	Sterol and fatty acid synthesis	Human host	E	Parasitism	Sterols and fatty acids can readily be taken over from the host[23]
Corals	*Acropora* sp.	Cysteine synthesis	Coral symbionts	P, M	Symbiosis	Cysteine biosynthesis is provided by endosymbionts[24]
Plants	Mycoheterotrophs	Photosynthesis	Fungi	P, M, E	Parasitism	Photosynthetic requirements are met by exploitation of fungi[25–27]
Plants	Holoparasites	Photosynthesis	Green plants	P, M, E	Parasitism	Photosynthetic requirements are met by exploiting other plants[28–32]
Plants	Mycoheterotrophs	Plastid genes encoding photosynthesis	Fungi	M	Parasitism	Photosynthetic and chlororespiratory genes become redundant once fungus provides photosynthesis products[33–35]
Plants	Mycoheterotrophs; some parasitic plants	Endosperm in seeds	Fungi/green plants	P, E	Symbiosis/ Parasitic	Carbon resources needed for germination are provided by the fungus or the host plant[36]
Plants	Endophyte infected grasses	Defences	Fungal endophytes	E	Symbiosis	Fungal endophytes provide defences against herbivores[37]
Algae	Flagellates	Photosynthesis	Diet	E	Herbivory	Increased grazing efficiency by small flagellates has led to a reduction in cell size and chloroplasts[38]

(continued)

Table 2. (continued)

Phylum	Species	Trait lost	Resource provider	Evidence	Interaction	Description
Fungi	Endophytic fungi	Sexual reproduction	Plants	E	Symbiosis	Host provides nutrients, shelter and transmission success[37]
Fungi	Mycorrhizal fungus: *Gigaspora* sp.	Vitamin production	Endobacteria	M	Endosymbiont	Endosymbiont vitamin synthesis alleviates the need for *de novo* synthesis[39]
Fungi	*Malassezia globosa*	Fatty acid synthesis	Human and other vertebrate hosts	M, E	Parasitism	Fungus excretes lipases to digest host skin lipids[40]
Fungi	*Encephalitozoon cuniculi*	Mitochondria, citric acid cycle, fatty acid synthesis	Mammal hosts	M	Parasitism	Major products of energy metabolic pathways are provided by the host[41]
Fungi	*Enterocytozoon bieneusi*	Carbon, isoprenoid and lipid metabolism	Mammal hosts	M	Parasitism	Major products of energy metabolic pathways are provided by the host[42]
Fungi	Powdery mildew fungi	Anaerobic fermentation, glycerol synthesis, nitrogen assimilation	Plants	M	Parasitism	Major products of energy metabolic pathways are provided by the host[43]
Fungi	*Rhizopus microspores*	Reproduction	Endobacteria: *Burkholderia* sp.	E	Symbiosis	The fungus is dependent on bacteria for formation of sporangia and spores[44]
Fungi	Attine fungus	Pathogen removal	Ants	E	Mutualism	Fungal gardens are protected from pathogens by ants[45–48]
Fungi	Rust fungi	Nitrogen and sulphur assimilation	Plants	M	Parasitism	Rust fungi are dependent on the host for nitrogen and sulphur assimilation[49,50]
Archaea	*Thermofilum pendens*	Essential lipid component	*Thermoproteus tenax*	M	Commensalism	*T. pendus* depends on *T. tenax* for an essential lipid component[51]
Bacteria	*Mycoplasma genitalium*	Amino acid, nucleotide and fatty acid biosynthesis	Vertebrates, Invertebrates and plants	M	Parasitism	Major products of energy metabolic pathways are provided by the host[52]
Bacteria	*Aliivibrio salmonicida*	Chitin utilisation	Fish host	M	Parasitism	Chitin utilisation for attachment to host and nutrition is impaired due to redundancy when using fish as a host[53]
Bacteria	*Ca. Moranella endobia,* and *Ca. Tremblaya princeps*	Amino acids	Nested symbiotic bacterium	M	Symbiosis	Amino acids are provided by a nested endosymbiont community[54]
Bacteria	*Candidatus Glomeribacter gigasporarum*	Nutrient acquisition	Arbuscular mycorrhizal fungus: *Gigaspora margarita*	M	Endosymbiont	The fungal host provides carbon, phosphorous and nitrogen[55]

References for footnotes are listed in Appendix S1.

hold across unrelated animal lineages involved in chemosynthetic symbioses, and show all the hallmarks of convergent evolution (Dubilier *et al.* 2008). In the case of oligochaete worms, trait loss has been so extreme that their nephridia (i.e. excretory systems) have been reduced. This type of excretory reduction, previously only known to occur in aphids in which symbionts recycle their waste products, is new to other free-living animals (Woyke *et al.* 2006). Researchers are now working on uncovering the novel metabolic pathways enabling worms to lose such fundamental traits.

Other major traits, such as ability to reproduce, have likewise been lost via ecological interactions with other organisms. The blight fungus *Rhizopus microspores* is dependent on the presence of endobacteria (*Burkholderia* sp.) to form the sporangia and spores needed to reproduce. If the fungus is cured of its endobacteria, it is unable to differentiate and produces only hyphae (Partida-Martinez *et al.* 2007). In return, the endobacteria produces a phytotoxin that favours the spread of the fungus. Although the *Burkholderia* has remained independent enough to be cultured in the absence of the host, the host has foregone the ability to reproduce independently (Partida-Martinez *et al.* 2007). This is similar to the evolved dependence of the springtail *Folsomia candida* on the bacterial endosymbiont *Wolbachia* to produce viable offspring (Timmermans & Ellers 2009).

Compensated trait loss evolves repeatedly in similar ecological interactions

Another feature that stands out from Table 2 is that compensated trait loss tends to occur as replicated evolutionary events, i.e., the same ecological interactions evolve repeatedly with different partner species (e.g. Dubilier *et al.* 2008). This may be indicative of the strong predictive value of ecological interactions as drivers of trait loss.

This is clearly demonstrated in parasitoids: insects that have adopted a parasitic lifestyle in which larval development is completed within or on another arthropod host species. Although parasitic insect species are capable of utilising dietary carbohydrates to meet immediate energy demands (Jervis *et al.* 2008), the conversion of such carbohydrates to long-term storage in the form of lipids is impaired even in the presence of excess carbohydrates (Visser *et al.* 2010). The parasitoid lifestyle renders larval lipid synthesis redundant, probably through extensive host manipulation in which host lipid resources are increased and carried over by the developing larva (Visser & Ellers 2008). Parasitoid species are found in various insect families, including the Hymenoptera, Diptera and Coleoptera, and have numerous independent evolutionary origins. Yet, in each of these separate occasions, the parasitic interaction has resulted in the evolutionary loss of lipid synthesis (Visser *et al.* 2010).

Similar convergent evolutionary patterns of compensated trait loss are found in other symbioses, such as in mycoheterotrophy (Merckx & Freudenstein 2010), or sap-feeding insects with nutritional endosymbionts (McCutcheon *et al.* 2009). The transition from phototrophy to heterotrophy has occurred multiple times in higher plants, as well as in virtually all major algal lineages. In the algal lineage Chrysophyceae (i.e. small flagellates) alone, the loss of photosynthesis has been found at least five times (de Castro *et al.* 2009). Studying replicated events is especially valuable since comparing genetic, developmental and phenotypic changes across different populations or species is a powerful approach to assess the generality of existing hypotheses and observations (Elmer & Meyer 2011).

THE CONTRIBUTION OF COMPENSATED TRAIT LOSS TO SHAPING EVOLUTIONARY DYNAMICS OF ECOLOGICAL INTERACTIONS

Although compensated trait loss evolves as a consequence of co-evolution, once compensated trait loss has taken place, it can itself act as a driver of further co-evolutionary processes. In general, trait loss tightens the ecological relationship by increasing the dependency between partners. This implies that, although unnoticed at the phenotypic level, compensated trait loss may direct the dynamics of many symbiotic interactions by preventing interacting partners from splitting up. Therefore, it has the potential to increase the evolutionary stability of ecological interactions by driving evolution away from facultative interactions and towards obligatory symbiosis.

Evolutionary trajectories towards compensated trait loss

How can species make the transition from facultative interactions towards obligatory ones with full loss of function? A 'mixed reliance' strategy may be a key evolutionary step in compensated trait loss in general. For example, mycoheterotrophy (reliance on carbon via fungal source for nutrition) in plants has evolved repeatedly from autotrophy (Merckx & Freudenstein 2010), with an associated loss of photosynthesis. Although the evolutionary steps to full reliance on fungal carbon sources are not well-understood, recent work suggests that establishment of partial mycoheterotrophy (i.e. combination of reliance on carbon from photosynthesis and fungi) is a principal pattern in the evolution of mycoheterotrophy.

Similarly, in certain flagellates the establishment of a mixed reliance strategy (i.e. autotrophy and heterotrophy) is proposed as a key evolutionary step, as opposed to a direct shift from autotrophy to heterotrophy. Flagellates have been proposed to have lost (or lost the functioning of) intracellular chloroplasts as a result of selection for increased grazing efficiency on small ultramicro bacterioplankton. This potential food source, which cannot be consumed by larger flagellates, is thought to have driven a reduction in cell size, loss of chloroplasts and facilitated the differentiation from a mixotrophic strategy to obligate heterotrophy (de Castro *et al.* 2009).

Interestingly, some species may still be in the transition towards full loss of function. Some heterotrophic plant species show incomplete photosynthetic loss, and rely only partially (or at certain life stages) on host carbon (Selosse & Roy 2009; Eriksson & Kainulainen 2011). Studying these cases can be especially informative in unravelling the evolutionary trajectories involved in compensated trait loss, but the challenge will be to be able to recognise these early stages.

Evolutionary stability of interactions under compensated trait loss

Several factors have been proposed to promote the evolutionary stability of symbiotic interactions, including strictly vertical transmission of symbionts between host generations (Herre *et al.* 1999), controlling mechanisms that limit exploitation of one partner by another (Kiers *et al.* 2011), and one-to-one based host and symbiont species (Frank 1996). The effect of trait loss on the dynamics of symbiotic interactions is as of yet unexplored, but may be an underestimated factor in explaining the evolutionary persistence of co-evolved systems.

Knowledge about the consequences of compensated trait loss is also crucial because under global change ecological relationships are prone to break up (Tylianakis *et al.* 2008; Berg *et al.* 2010; Kiers *et al.* 2010). Without a partner providing the necessary resource or function, species will be exposed to the detrimental fitness effects of their trait loss. Habitat loss, climate change or increased competition by invading species resulting in the loss of one species may trigger coextinction of the species that depends on it (Dunn *et al.* 2009), unless a new partner can be found that provides a similar service. A recent review identified mutualisms with strict dependence as among the least resilient to current anthropogenic change (Kiers *et al.* 2010). Nearly all cases of compensated trait loss listed in Table 2 involve dependence on a specific partner species, which would make ecological interactions with compensated trait loss highly vulnerable.

Strict dependence on the ecological partner could be mitigated if new relationships can be created with other ecological partners, even after compensated trait loss has taken effect. The likelihood of such a scenario may vary for different ecological interactions. For example, in specialised parasitic interactions host switching is a rare event, but for endosymbionts there is limited evidence for partner switching. In the pea aphid, facultative endosymbionts have been shown to effectively replace the essential symbiont in an experimental setup (Koga *et al.* 2003). In curculionid weevils more recently acquired symbionts have entirely replaced ancestral symbionts (Lefevre *et al.* 2004), but it is unknown if trait loss has occurred in this case. This work suggests broader community interactions may offer a way to switch to relationships with new partners. A more likely scenario may be that multiple symbionts are permanently established, with the newly acquired symbiont being complementary to the more ancient symbiont (Perez-Brocal *et al.* 2006; Lamelas *et al.* 2011). Similar multi-partner symbiont communities have been found in the fungal symbionts of beetles (Bleiker & Six 2009) and in ant-plant mutualisms. *Acacia* plants sequentially associate with four different ant species that synergistically act to increase life time host fitness (Palmer *et al.* 2010). The crucial question is how fast new alliances can evolve. If extinctions are occurring at an accelerated rate, will the evolutionary response be fast enough to create new relationships to compensate? For long-lived, slow-evolving species such a scenario seems unlikely. In these cases, the only possibility to escape coextinction would be to re-evolve the lost trait.

Reversal of compensated trait loss

Is it possible to re-evolve a lost trait? Although it is often believed that trait loss is irreversible because of mutation accumulation in the affected genes (known as Dollo's law), several studies show the

lost phenotype can be readily regained (e.g. Cruickshank & Paterson 2006; Bely & Sikes 2010). Compensated trait loss has been reversed for instance in the case of parasitic insects, in which several species have regained the ability for lipid synthesis (Visser *et al.* 2010). The ecological and molecular underpinnings of such trait reversal are currently unknown (Collin & Miglietta 2008; Porter & Crandall 2003). The types of mutations that lead to trait loss have important implications for the potential reversal of trait loss. For example, reversion of regulatory changes could explain the ease with which some organisms regain lost traits, such as the ability for lipid synthesis in parasitic insects (Bely & Sikes 2010).

Adding to the complexity is the notion that some cases that we regard as trait loss, are actually reversals of earlier symbioses (i.e. acquisition of organelles such as chloroplasts and then subsequent loss in parasitic plants). In these cases, the symbiosis has been established for so long that we have come to consider them as traits of the organisms themselves. For example, the ability to form the arbuscular mycorrhizal symbiosis is considered the 'ancestral state' of land plants, and thus non-mycorrhizal plants are considered the derived state, in which a trait has been lost (Wang & Qiu 2006), even though this can be considered a reversal of an earlier symbiosis. Comparative phylogenetic studies are required to ascertain if trait loss reversal is a general feature of compensated trait loss.

WAYS IN WHICH RAPIDLY EMERGING GENOME SEQUENCE AVAILABILITY WILL INCREASE OUR KNOWLEDGE ON COMPENSATED TRAIT LOSS

Genomic information can provide a strong foundation for studying patterns of compensated trait loss. It helps enable a more precise determination of the ecological roles of close interacting partners, beyond traditional phenotypic characterisation. In some cases, such as in endosymbiotic bacteria, genome sequencing has been the only method for identifying the role each partner plays in the interaction. With the recent advances in DNA sequencing technology and the resultant increase in genome sequence information, this approach is also becoming an increasingly useful tool for identifying cases of eukaryote trait loss. We propose three major approaches that use molecular tools to study compensated trait loss that will help in advancing the field forward.

Comparative genomics enables quantitative analyses of compensated trait loss

Comparative genomics across broad lineages of organisms is perhaps the most comprehensive tool for identifying potential cases of cryptic or incipient trait loss. For example, it is possible to determine the presence and absence of genes associated with essential metabolic pathways across all sequenced genomes. Identifying such gene loss signatures within specific taxa is a useful tool in compensated trait loss research because it means that organisms must be relying on reliable sources from which to acquire the products normally produced by those pathways.

Utilising the manually curated genome annotations from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, we conducted a simple analysis to assess the potential for broad genomic comparisons to identify signatures of compensated trait loss (Box 1). Our broad screen of genome content not only easily identified well-known cases of trait loss in bacteria, but also identified lesser-known, but significant losses within some other genera of bacteria (e.g. *Treponema* and *Streptococcus*, Fig. 2a). Interestingly, we found relatively more extensive trait loss in pathogenic protists and some fungi, as compared to free-living eukaryotes. Also, we found more gene loss – suggestive of compensated trait loss – in metazoans than plants and fungi (Fig. 2b). Our analysis provides an estimate of enzymatic reaction loss across the available complete genomes. The capability to compare genomes from both closely related organisms and across entire domains has shown great promise in other fields of biology (Aravind *et al.* 2006; Crespi *et al.* 2010; Elmer *et al.* 2010), and the complete genome collection will become an increasingly powerful tool for identifying compensated trait loss as it continues to expand beyond model organisms and pathogens.

The quantitative analysis above provides a glimpse at how genomic databases can be utilised to identify larger patterns in trait loss dynamics. As more and more genomes become available, comparative studies among organisms involved in various types of ecological interactions are an obvious way forward (Kleiner *et al.* 2012). New tools and databases are being introduced to facilitate comparative genomic analyses. For example, Zhang *et al.* (2010) recently developed a pipeline to identify functional gene losses in the human genome, using the absence of human orthologs compared to their mouse counterparts as the signature of pseudogenes, i.e. disabled genes without a functional copy. A particularly valuable advantage of this novel method is that the timing of separate gene loss events can be estimated through comparison with other mammalian genomes (Zhang *et al.* 2010). Complete genomic information is there-

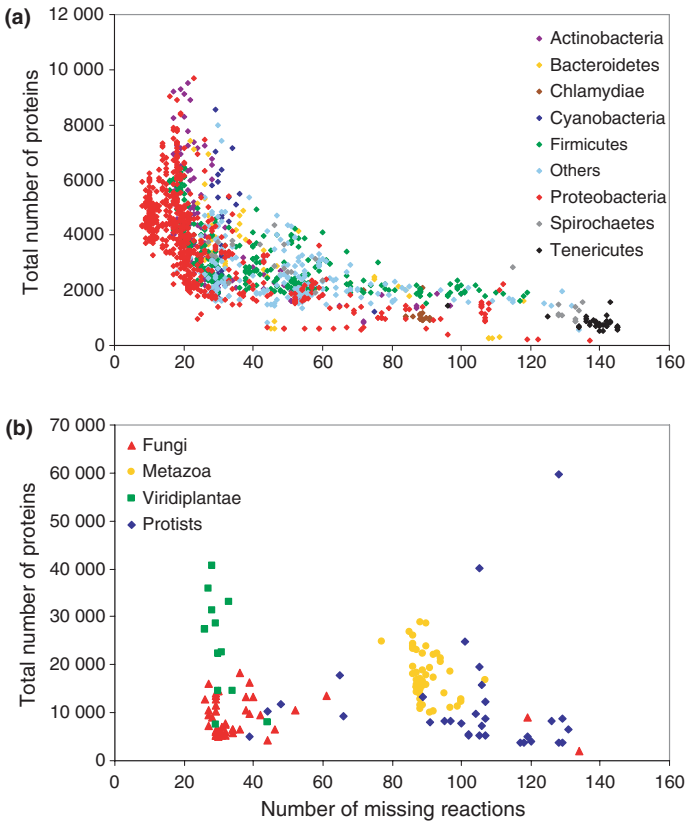

Figure 2 Relationship between the total number of predicted proteins contained in a genome and the number of enzymatic reactions absent from a number of essential pathways we selected from the KEGG database. Prokaryote (a) and eukaryote (b) taxa are colour-coded by phylum.

fore necessary for phylogenetically related species, in addition to the species in which gene loss is studied. A further use of genomic data is to investigate changes in regulatory programmes associated with trait loss. By focusing on related species that either have experienced or have not experienced compensated trait loss, researchers can make comparisons of the regulatory effects of many, even thousands, genetic changes in a single experiment (Tirosh & Barkai 2011). This focus on divergence of regulatory programmes will be key in identifying the underlying molecular mechanisms of trait loss.

The current small set of model organisms needs to be extended to apply large scale-genomic approaches as outlined above. The goal is to move towards a larger set of diverse organisms experiencing trait loss, and their phylogenetic relatives to enable comparative studies. Many of these species are notoriously hard for genomic studies (e.g. arbuscular mycorrhizal fungi), yet are likely to unearth incredibly interesting findings. The good news is that this work is becoming faster and cheaper, which will facilitate rapid advances.

Genome sequence availability enhances phylogenetic resolution

How can we effectively use phylogenetic tools to detect compensated trait loss? In practice, this involves testing whether or not the loss of a trait is dependent on interactions that have evolved with another organism (Maddison 1990). Various approaches can be utilised to study such correlated trait evolution, allowing the reconstruction of lineages to determine how dependency arises. New techniques allow for these processes to be studied at high resolutions, even at the level of proteins. For example, phylogenetic methods are emerging with the goal of detecting co-evolved, interacting protein networks (Juan *et al.* 2008). These methods enable scientists to look for direct compensatory changes at the molecular level.

These methods can be used in concert with traditional mapping approaches (e.g. Machado *et al.* 2001) that have been critical in exploring why particular traits are lost. Recent studies have argued for the concert use of multiple approaches when testing for trait loss to ensure robust conclusions (Syme & Oakley 2012). One particularly promising way forward is the emerging field of Ecophylogenetics (e.g. Mouquet *et al.* 2012), which advocates the use of phylogenetic data to assess the likelihood of alternative scenarios of community assembly. Such approaches, which successfully merge the disciplines of ecology, biogeography and macroevolution, can potentially be used in a predictive manner (i.e. 'phylogenetic diagnostic' tools) to ask how closely species need to interact before we see a degradation of traits.

Increasing the resolution of phylogenetic studies will ultimately allow us to focus on larger questions, for example understanding how compensated trait loss modifies changes in speciation rates by comparing lineages showing variation in extent of trait loss. A greater understanding of how trait loss contributes to co-evolutionary process will aid predicting how lineages may respond to future change.

Predictive modelling

To fully utilise the data provided by genomic sequencing, new models must also be created. Ecology, Evolution, Systems Biology, and many other fields have benefited enormously from utilising new modelling tools (Lauro *et al.* 2009; Röling *et al.* 2010). A promising approach is NetSeed, an open-source tool that allows researchers to analyse the evolution of microbes and their environments by focusing on topologies of metabolic networks (Carr & Borenstein 2012). This approach has been called 'Reverse Ecology' and relies on algorithms for predicting microbial metabolic networks. This permits the quantification of an organism's metabolic dependence on its environment/other microbes (termed 'seed set'), and enables high-throughput genomic data to be translated into large-scale ecological data (Carr & Borenstein 2012). Such tools allow researchers to calculate metabolic overlap between organisms (Freilich *et al.* 2010), potentially facilitating the identification of trait loss.

Classical quantitative genetics analysis can also contribute to predictive modelling, especially when combined with individual-based model simulations to study multidimensional trait co-evolution in hosts and parasites. The advantage of this approach is that it allows researchers to study what conditions favour particular combination of traits (Gilman *et al.* 2012), and potentially the conditions that favour trait loss. A similar approach uses game theory modelling, a method for analysing strategic behaviour over evolutionary time, to determine the specific conditions under which it is advantageous for an organism to stop performing a function (Morris *et al.* 2012). Dynamic modelling has been used to test competing heterotrophy and mixotrophic strategies in flagellates to determine when trait loss is advantageous. This allows researchers to theoretically test different parameters (e.g. carbon concentration, light availability) driving the loss of photosynthesis (de Castro *et al.* 2009). Predictions can then be tested using experimental evolution approaches under controlled environmental selection pressures. Such combination between model predictions and empirical tests will be a key step in refining our predictive ability for compensated trait loss.

CONCLUSIONS

Although studies of compensated trait loss have investigated a large range of organisms, there are few, if any, comparative studies that focus on patterns across lineages. More research is needed to determine predictability of patterns across diverse species and to derive general evolutionary principles. Do the same ecological interactions lead repeatedly to trait loss within different partner species? Well-studied examples could be utilised to inspire targeted research into new systems. For instance, fungus farming in leaf-cutter ants has led to compensated loss of arginine biosynthesis pathway in two separate genera (Suen *et al.* 2011; Nygaard *et al.* 2011). Other cases of fungus farming, for example in termites or bark beetles (Bleiker & Six 2007), would therefore be obvious candidates to find compensated trait loss. Also other farming systems, such as husbandry in *Littoraria* snails (Silliman & Newell 2003), slime moulds (Brock *et al.* 2011) and *Stegastes* damselfish (Hata & Kato 2006) are potentially prone to compensated trait loss. As a prelude to future work, we identified several cases in which we would expect compensated trait loss, but which have not been studied in sufficient detail (Table 3). This type of targeted research will be useful in identifying the commonalities driving compensated trait loss across different species.

Lastly, studies of compensated trait loss may be utilised in applied ways. This may be particularly applicable for studies of pathogenic fungi. The blight fungus *R. microspores* is responsible for severe losses in rice systems. However, because it is dependent on the presence of endobacteria to form the sporangia and spores needed to reproduce (Partida-Martinez *et al.* 2007), curing the fungus of the endobacteria might stop or slow its spread. Researchers should

Table 3 Potential cases of compensated trait loss

Phylum	Species	Hypothesised trait lost	Resource provider	Interaction	Description
Molluscs	*Littoraria inundata*	Digestive capabilities	Fungal farming for nutrients	Symbiosis/ Predation	Snails graze live marsh grass to prepare substrate for fungal growth and consume invasive fungi[1]
Insects	Bark beetles	Nutrient acquisition	Symbiotic fungi	Symbiosis	Beetles inoculate trees with fungal spores; fungal mycelia curtail tree defences and/or serve as beetle food[2,3]
Insects	*Asteromyia carbonifera*	Loss of sterol synthesis	Galling fungus	Symbiosis	Fungal mycelia form food source for the midge; midge larvae obtain sterols mainly from fungus[4,5]
Insects	Lycaenid butterflies	Larval mobility	Various ant species	Symbiosis/ Parasitism	Ants carry butterfly larvae to nest and tend them[6]
Plants	Orchids	Germination	Mycorrhizal fungus	Symbiosis	Mycorrhizal fungus is vital for successful germination, growth and establishment[7]
Fungi	Fungi	Spore dispersal ability	Pollinating insect	Symbiosis	Fungus transforms plant morphology to attract pollinators, which spread fungal spores[8]
Algae	*Polysiphonia* sp.	Competitive ability	Damselfish: *Stegastes nigricans*	Mutualism	Fish maintain *Polysiphonia* monoculture by removing algal competitors[9]

References for footnotes are listed in Appendix S1.

focus on cases in which one partner can be manipulated, either to control or favour the spread of the other, to our benefit. Likewise, compensated trait loss research can provide an important body of knowledge for species conservation. This is increasingly important in situations of tight co-evolution, where the loss of one species can result in a cascading loss of others (Kiers *et al.* 2010). Organisms experiencing compensated trait loss embedded in complex communities may be particularly fragile to disturbance. Future work should aim to understand how compensated trait loss influences the vulnerability of species to change.

Box 1

To explore the potential of large-scale comparative genomics for identifying signatures of compensated trait loss, we determined the presence and absence of a number of essential genes across 1376 bacterial and 141 eukaryote genomes (DataS1 and S2). Specifically, we selected a set of pathways defined by the KEGG database for the biosynthesis of several essential small molecules, including 12 amino acids, nucleotides, haem, and fatty acids (Appendix S2). The number of enzymatic reaction steps in each pathway that were identified in the KEGG annotation of the genome was determined for each organism. As with any database-centric methodology, our analysis is limited by the pathway annotations in KEGG. Because of this, it is important to recognise the limitations of this approach for less well-characterised organisms, such as Archaea, which may have alternative pathways not annotated by the database. The complete genome collection is also biased towards pathogens and model systems, particularly in eukaryotes. Despite these limitations, the KEGG database provides a relatively simple, consistent framework for identifying trends across all complete genomes.

As expected, genera of bacteria which are known to be strictly host-associated such as *Buchnera*, *Borrelia, Wolbachia, Chlamydia* and *Mycoplasma* were among the bacterial genera that contained fewest numbers of enzymatic reactions from the pathways we selected. In contrast, some facultatively host-associated bacterial genera did not show this trend, such as *Escherichia* and *Salmonella*. These genera were among those with the largest number of reactions from the KEGG pathways we selected, reflecting their metabolic versatility. We also found a surprising amount of variation in the number of these reactions within several genera. Within the seven complete genomes in the genus *Treponema*, the two sequenced strains that cause syphilis and an oral pathogenic strain on average contained 70 fewer reactions on average than those strains found in animal guts. This fits predictions based on compensated trait loss; the pathogens have a highly specific and co-evolved intimate connection with their hosts while those strains found in the animal gut are embedded in a diverse community of microbes. There was also significant variation in the number of reactions present within the genera *Lactobacillus* and *Streptococcus,* although the ecological differences that may have led to this variation are less clear since many of the sequenced strains have some ability to cause infection. Nevertheless, the relative number of putatively essential biosynthetic genes lost in a strain may be one method to distinguish obligate vs. opportunistic pathogens within these genera.

Overall, the bacteria and Archaea showed a strong correlation between total number of proteins contained in a genome and the number of enzymatic reactions absent (Fig. 2a). The two phyla with the largest number of genomes, the Proteobacteria and the Firmicutes, had genomes across the spectrum of reaction loss. Other phyla, such as the Chlamydiae and the Tenericutes, were clustered within a more narrow range of reaction losses.

The trend of trait loss in obligate pathogens also held true in eukaryotes, in which parasitic protists such as *Plasmodium* were lacking a larger number of reactions than free-living organisms (Fig. 2b). There was also a clear distinction between plants and fungi and metazoans, with plants and fungi generally missing between 20 and 45 reactions, while the metazoans were missing between 80 and 100 reactions. The two major exceptions to this general trend were two fungi, the brown rot fungus *Postia placenta* (119 missing reactions) and an obligate intracellular fungal pathogen, *Encephalitozoon cuniculi* (134 missing reactions). The two primary outliers within the metazoa were the platypus (107 missing reactions) and the sea anemone *Nematostella vectensis* (77 missing reactions).

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AUTHOR CONTRIBUTIONS

JE designed the study, all authors collected relevant literature, BM performed the KEGG analysis and all authors contributed substantially to writing the manuscript.

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