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Review

Symbiosis as a source of selectable epigenetic variation: taking the heat for the big guy

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Evolutionary developmental biology is based on the principle that evolution arises from hereditable changes in development. Most of this new work has centred on changes in the regulatory components of the genome. However, recent studies (many of them documented in this volume) have shown that development also includes interactions between the organism and its environment. One area of interest concerns the importance of symbionts for the production of the normal range of phenotypes. Many, if not most, organisms have 'outsourced' some of their developmental signals to a set of symbionts that are expected to be acquired during development. Such intimate interactions between species are referred to as codevelopment, the production of a new individual through the coordinated interactions of several genotypically different species. Within the past 2 years, several research programmes have demonstrated that such codevelopmental schemes can be selected. We will focus on symbioses in coral reef cnidarians symbiosis, pea aphids and cactuses, wherein the symbiotic system provides thermotolerance for the composite organism.

Keywords: symbiosis; mutualism; eco–devo; group selection

1. INTRODUCTION

> Then [de Bary, 1879] came to a central reason for his lecture – symbiosis was a major source of evolutionary novelty that could and should be investigated experimentally.
>
> (Sapp 1994; p. 9)

The theory of evolution by natural selection is predicated on the existence of widespread variation within species. But from whence does this variation arise? Darwin (1859) realized that selection could not act upon characters that had not yet appeared, noting that 'characters may have originated from quite secondary sources, independently from natural selection'. He continued this line of reasoning in his book *The variation of animals and plants under domestication* (Darwin 1868), where he concludes (p. 351, in his discussion of the origin of nectarines from several different varieties of peach, each in a different environment), 'the external conditions of life are quite insignificant, in relationship to any particular variation, in comparison with the organization and constitution of the being which varies. We are thus driven to conclude that in most cases the conditions of life play a subordinate part in causing any particular modification . . .'.

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One contribution of 12 to a Theme Issue 'From polyphenism to complex metazoan life cycles'.

There are genetic and epigenetic sources of hereditable variation. Population genetics provided the first set of answers to Darwin's quandary. Alleles of the protein-encoding regions of the genome were shown to be major sources of variation. Mutations of human globins, for instance, were seen as providing variation that was selectable and which produced organisms with different fitness in the population (Gilbert & Epel 2009). Such variation was seen to be 'Darwin's missing evidence' (Kettlewell 1959). Later, developmental genetics found that there were other sources of variation, which resided in the nucleotide sequences outside the coding regions of the genes. These developmental genetic mechanisms of variation include heterochrony (change in the timing of gene expression), heterotopy (change in the cells in which genes are expressed) and heterometry (change in the amount of gene expression)[1]. Examples have been found for each of these mechanisms (Arthur 2004; Gilbert & Epel 2009).

But development is larger than just developmental genetics; for there are also environmentally induced components of developmental variation. These include developmental plasticity, developmental symbioses and epialleles caused by environmentally induced chromatin modification. It is therefore important to determine whether these environmental mechanisms produce selectable variation. The selectability of epialleles and developmental plasticity has been discussed by Jablonka & Lamb (1995), Jablonka & Raz (2009) and by West-Eberhard (2003, 2005).

Moreover, Margulis (1991, 1998) has detailed the importance of symbiosis between adult organisms. This paper will be a more focused review on developmental symbiosis and its possible role in providing selectable variation.

2. DEVELOPMENTAL SYMBIOSIS

Darwin's (1859) idea of the 'struggle for existence' where competition exists between 'one individual with another of the same species or with the individuals of distinct species' sets up a framework where each individual is essentially singular, competing only for itself and the survival and propagation of its lineage. But this situation changes if the 'individual' is actually a 'team' or a 'consortium' of cells with different genotypes. Gilbert (2002) referred to this chimeric mode of development as 'interspecies epigenesis,' emphasizing the developmental roles played by symbionts and the notion that the fertilized egg is not an autopoietic, self-creating, entity. Rosenberg *et al.* (2007) referred to this phenomenon of variation through symbiosis as 'the hologenome theory of evolution'. They referred to the host and its full symbiont population as the *holobiont*, and they named the combination of the host genome and the genomes of all its symbiotic organisms the *hologenome*. However, this original hologenome concept did not include development as an aspect, and we would like to expand it to include not only symbiosis but also *symbiopoiesis*—the codevelopment of the holobiont.

More and more, symbiosis appears to be the 'rule,' not the exception (McFall-Ngai 2002; Saffo 2006; Gilbert & Epel 2009). One well-studied example of developmental symbiosis is in the Hawaiian bobtail squid *Euprymna scolopes*. A tiny (5 cm long) native of the shallow waters off Hawaii, this nocturnal animal preys on shrimp and runs the risk of alerting predatory fish to the squid's presence if the moon casts the squid's shadow as it skims across the water overhead. *Euprymna scolopes*, however, has developed a mechanism of dealing with this potential threat: it emits light from its underside, mimicking the moonlight and hiding its shadow from potential predators. The squid cannot accomplish this feat alone; the presence of the symbiotic bacterium *Vibrio fischeri* in the squid's light organ is required to generate the squid's characteristic glow. Both the squid and the bacterium benefit from this mutualistic relationship. The squid gains protection from predators and the bacterium is able to live safely within the host's light organ, an environment free of predators and adverse environmental conditions. More significantly, *V. fischeri* actually constructs the light organ in which it will reside. The newly hatched squid collects bacteria from the sea water. Only members of the species *V. fischeri* are allowed to adhere to the underside of the squid and to induce apoptosis in the tissue that will become the light organ. And only when they have reached a certain density do they begin to emit light (Nyholm & McFall-Ngai 2004; Visick & Ruby 2006).

The development of numerous insects involves obligate symbiosis with bacterial partners, and several species of insects cannot develop properly if certain symbionts are removed. In the parasitic wasp *Asobara tabida*, normal female development is dependent on *Wolbachia* infection. If *A. tabida* females carrying *Wolbachia* are treated with antibiotics that kill the symbiotic bacteria, the female wasps are unable to produce mature oocytes, and thus cannot reproduce. In fact, without the bacteria, the ovaries undergo apoptosis (Dedeine *et al.* 2001; Pannebakker *et al.* 2007). Unlike the squids, which receive their symbionts 'horizontally' from the sea water, *A. tabida* infects its juveniles 'vertically' through the egg cytoplasm. Thus, *Wolbachia* bacteria has become an epigenetically transmitted source of developmental signals. *Asobara tabida* has become dependent on the presence of *Wolbachia* for successful reproduction.

In plants, symbiosis is responsible for nitrogen fixation, and endophytic fungi have been found in every plant species examined. Some of these endophytes provide protection—a very common function of symbionts (Gilbert & Epel 2009). In the rye grass *Achnatherum robustum*, an endophytic fungus produces a compound that prevents insects and nematodes from eating the grass (Faeth *et al.* 2006). Mycorrhizal fungi are also common throughout the plant world. The beauty of the orchid is owing largely to such fungi. Orchid seeds contain no energy reserves. An orchid may produce thousands of seeds, but only those that find a fungal partner can get the carbon they need to germinate (Waterman & Bidartnando 2008). Symbiosis is a major player in the evolutionary game. It is not just for lichens.

The host and symbiont species interact in ways that are vital to the proper functioning of both organisms. Disruption of that interaction can lead to illness and death. For example, Mazmanian *et al.* (2005) showed that mice raised without gut microbiota have deficient proliferation of helper T cells, but the introduction of *Bacteroides fragilis* to the gut was enough to stimulate T-cell expansion. They were also able to demonstrate that *B. fragilis* protects mice from experimental bowel colitis normally induced by a second symbiotic bacterium, *Helicobacter hepaticus* (Mazmanian *et al.* 2008). In exchange for these benefits, the mice provide the bacterium with a relatively safe and nutrient-rich environment. This has important medical implications for the health of humans, considering that the human digestive tract harbours over 500 species of bacteria (Gilbert & Epel 2009).

These symbionts and hosts do not lead independent existences. Rather, they are the mutual cause of the other's development. The *Bacteroides* in the mammalian gut induces the expression of genes in the intestinal epithelium, resulting in the proper development of the mammalian gut, gut vasculature and host immune system (Hooper *et al.* 2001; Rhee *et al.* 2004). We have outsourced certain developmental signals to these bacteria, and if they are not present, our bodies do not finish normal development. We live in complex relationships with our symbionts. The above-mentioned *Bacteroides*, for instance, induces gene expression in the murine intestine, instructing the Paneth cells to produce angiogenin-4 and RegIII. These proteins provide benefits to both the mammalian body and the *Bacteroides*. Angiogenin-4 helps

induce blood vessel development in the villi, and angiogenin-4 and RegIII are each selective bacteriocidal proteins that kill competitors of *Bacteroides*, such as *Listeria* (Hooper *et al*. 2003; Cash *et al*. 2006). In inducing gene expression in its host's intestinal epithelium, *Bacteroides* does well by doing good. It helps construct its own niche by creating mutually favourable conditions in the gut (Laland *et al*. 2008). In return, our intestinal cells instruct the bacteria to produce biofilms, allowing the bacteria to continue residence therein. Thus, as expected in development, there are reciprocal inductions. Only here, they are between different species residing in the same body. Kauffman (1995) famously said that 'All evolution is coevolution.' The situation may actually be more intimate. Almost all development may be codevelopment. By codevelopment we refer to the ability of the cells of one species to assist the normal construction of the body of another species.

3. CODEVELOPMENT AND NATURAL SELECTION

It has been proposed that symbiotic relationships are unstable over evolutionary time, and thus are both rare and evolutionarily transient, because organisms with genotypes that confer advantages to non-kin are at a disadvantage in comparison with organisms with 'selfish' genotypes that do not provide other species with such benefits (Douglas 2007). However, the persistence of symbioses such as the coral–algae symbiosis that evolved approximately 240 Myr ago, and continues to this day, seem to indicate otherwise. Most symbiotic relationships involve microorganisms that have fast growth rates and can thus change more rapidly under environmental stresses than invertebrates or vertebrates. Rosenberg *et al.* (2007) describe four mechanisms by which microorganisms may confer greater adaptive potential to the hologenome than can the host genome alone. First, the relative abundance of microorganisms associated with the host can be changed due to environmental pressure. Second, adaptive variation can result from the introduction of a new symbiont to the community. Third, changes to the microbial genome can occur through recombination or random mutation, and these changes can occur in a microbial symbiont more rapidly than in the host. Fourth, there is the possibility of horizontal gene transfer between members of the holobiont.

In a symbiotic relationship, the interactions among partners can affect the evolutionary fitness of both the symbiont and the host. While the genomes of the individual symbionts affect the development of each organism, development of symbiotic species is also regulated by interactions of the symbiont genomes within the holobiont (Ley *et al.* 2005; Gilbert & Epel 2009). This in turn could alter the fitness of the organisms involved in the symbiosis, which would make the symbiotic relationship a powerful evolutionary force. In this sense, the individual is actually a community of organisms behaving as an ecosystem. In group selection theory, the group is usually treated as an individual. Here, the individual is treated as a group. Nature may be selecting 'relationships' rather than individuals or genomes. What we usually consider to be an 'individual' may be a multispecies group that is under selection.

If the relationship between symbiotic species is so important, then perhaps the environment selects not only on each species in the relationship, but also among variants of the holobiont. The fitness traits would therefore be not merely those of the host, but the traits of the group *per se*. Therefore, it may prove useful to look at the evolution of a single genome in the context of the hologenome. This view of evolution would link these species together as a single coevolving unit, because the fitness of each species would rely on its interactions with the other species in the symbiosis. The three cases presented in this paper focus on the thermotolerance of hologenomes due to changes in one of the genomes in the symbiosis. These cases include corals with zooxanthellae, pea aphids with the bacteria *Buchnera aphidicola*, and Christmas cactuses with two types of fungi, *Chaetomium chiversii* and *Paraphaeosphaeria quadriseptata*. The coral/zooxanthellae symbiosis shows how the fitness of the hologenome in warm waters is improved by altering the relative abundance of symbiont populations. The pea aphids and *B. aphidicola* are examples of how a genetic mutation in a bacterial genome can confer heat tolerance to the entire holobiont. The example of Christmas cactuses and their fungi demonstrates how heat tolerance can be achieved through interspecies signalling and provides a possible pathway through which heat tolerance can be shared. In all three cases we can observe that evolution is acting or has acted upon the relationships between the species, both host and symbionts, adapting them as a unit to each other and to their selective environments.

4. CORAL AND *SYMBIODINIUM* SYMBIOSIS: MARINE THERMOTOLERANCE

Symbiodinium is a genus of photosynthetic endosymbiotic dinoflagellates. The genus comprises multiple species of zooxanthella algae, which have been found to inhabit the tissues of scleractinia coral. Scleractinia, also known as Stony Corals, makes up most of the framework of coral reef structures found in shallow tropical waters. All Scleractinia have hard skeletons and can be divided into two separate sub-groups: colonial corals that build reefs and solitary corals that do not. Reef-building scleractinians can be further divided into two types: hermatypic and ahermatypic. Ahermatypic corals are heterotrophic and feed on plankton whereas hermatypic corals rely on endosymbiotic *Symbiodinium* for 95 per cent of their energy supply (Muscatine 1990). Hermatypic coral are therefore largely dependent on their endosymbionts for survival and in return provide the zooxanthella with protection, nutrients and a supply of carbon dioxide for photosynthetic products. Under stressful environmental conditions, corals undergo a bleaching event where they expel or digest their endosymbiont populations, leaving behind a white skeletal structure. Such events have increased in recent decades and are expected to occur more frequently in the near future due to global warming (Hoegh-Guldberg *et al.* 2007).

Within the *Symbiodinium* genus there exists a great deal of genetic and physiological diversity. Eight clades have been identified, six of which form relationships with corals (Baker 2003). The clades are classified A–H by analysis of nuclear ribosomal and chloroplast DNA (Baker 2003; Pochon *et al.* 2006). *Symbiodinium* can differ in traits such as the photosynthetic response to light and thermal tolerance (Robinson & Warner 2006). For example, Clade D zooxanthellae are less heat sensitive in comparison with Clade C zooxanthellae and can thus tolerate higher temperatures (Fabricius *et al.* 2004). Even subtle genetic differences among subcladal *Symbiodinium* populations have been shown to influence coral bleaching susceptibility (Sampayo *et al.* 2008).

Although genetically distinct coral colonies can have unique zooxanthellae DNA fingerprints (Goulet & Coffroth 2003), real-time polymerase chain reaction methods that can detect background symbionts at levels as low as 0.001 per cent have shown that most coral colonies harbour multiple strains of *Symbiodinium*. These techniques have shown that coral colonies from four scleractinian species (*Acropora millepora*, *Acropora tenuis*, *Stylophora pistillata* and *Turbinaria reniformis*) previously thought to harbour only a single *Symbiodinium* clade actually harbour multiple strains (Berkelmans & van Oppen 2006; Mieog *et al.* 2007). Moreover, this ability to house multiple strains may enable the changes in such symbiotic zooxanthellae, which provide protection against thermally induced bleaching.

The ability to support several different clades of *Symbiodinium* in one host colony has led to theories of 'symbiont shuffling' (Baker 2003; Goulet & Coffroth 2003; Goulet 2006). Here, the resident *Symbiodinium* algae can compete with one another and create a new combination of the coral and zooxanthellae hologenome from strains that are already within the coral. Low level background symbionts have the ability to outcompete the dominant clade given the right environment (Baker 2003). With symbiont shuffling, no new symbionts are introduced from the environment. Rather, the environment places selective pressure on the different types of *Symbiodinium* cells already within the coral tissue. Berkelmans & van Oppen (2006) have evidence that such symbiont shuffling between clades of *Symbiodinium* can occur in transplanted populations of *A. millepora* in the Great Barrier Reef. The corals originally have a large population of type C *Symbiodinium* and minor populations of type D. Once the faster reproducing type C symbionts were expelled from the corals during heat stress, the thermally tolerant type D zooxanthellae were able to dominate in that particular colony of *A. millepora* transplants.

Moreover, when the surviving *A. millepora* population changed the symbiont from type C to type D, their thermal tolerance and photosynthetic yields increased appreciably. It is suggested that the thermal tolerance of zooxanthellae relies heavily on the stability of the thylakoid or other lipid membranes of their chloroplasts. The more stable the thylakoid membranes, the higher the concentrations of unsaturated fatty acids and the less vulnerable host tissues are to attack by reactive oxygen molecules (Berkelmans & van Oppen 2006). It is hypothesized that the thermally tolerant D strain of zooxanthellae possess more stable thylakoid membranes that enables it to cope better with rapid rates of global warming (Tchernov *et al.* 2004).

Alternatively, other investigations have proposed that 'symbiont switching' could be the major way of changing the dominant population of endosymbionts. Symbiont switching is achieved through the elimination and replacement of the dominant clade of *Symbiodinium* by a new strain of endosymbionts from the surrounding environment. The environment selects which cells survive within the body. While the above-mentioned experiments supported the symbiont shuffling hypothesis, a subsequent study in 2008 by the same researchers provided evidence for symbiont switching among corals that did not appear to contain a minor, more heat tolerant, population of *Symbiodinium* (Jones *et al.* 2008). This may have important ecological consequences, since symbiont shuffling to more heat-resistant types may not be efficient enough to keep up with global climate change and it may not be possible for many species. Over the next 100 years, it is predicted that average tropical sea temperatures will increase by 1–3°C (Berkelmans & van Oppen 2006). Therefore, in order to adapt to the changing environment, coral colonies would greatly benefit from evolving a method of symbiont shuffling or switching.

5. FUNGUS-CONFERRED HEAT TOLERANCE IN DESERT PLANTS

Whether in response to drought, disease or heat shock, plants have been outsourcing parts of their stress response to fungal infections for perhaps 400 Myr (Rodriguez & Redman 2008). In several cases, holobionts can survive harsh conditions that neither partner could survive alone (Márquez *et. al.* 2007; Rodriguez & Redman 2008). However, in almost none of these cases is the mechanism or signalling of such interactions well understood.

One promising pathway that is now being explored involves heat shock proteins (HSPs). HSPs have been shown to play roles in stress-response (heat-induced and otherwise), protein folding and protein degradation, among others. HSPs are grouped into families based on their approximate size in kilodaltons (i.e. an HSP-90 is approximately 90 kDa). Separate families of HSPs have different functions under different cellular conditions, and they are capable of self-regulation (DiDomenico *et al.* 1982). As a result, certain HSPs may regulate one another's expression and thereby transcribe at different levels in response to different stresses. HSPs can even inhibit the expression of one or more HSPs. For example, HSP-90 has been shown to inhibit the expression of two particular HSPs required for plant thermotolerance (Gurley 2000; Queitsch *et al.* 2000): HSP-70 and HSP-101 (McLellan *et al.* 2007).

HSP-90 comprises 1–2% of all proteins in the cytosol of unstressed cells (Chen *et al.* 2006), and up to 4–6% of heat-stressed cells (Crevel *et al.* 2001).

The family of proteins is highly conserved (around 55%) between species as far removed as yeast and humans (Chen *et al.* 2006). HSP-90 is known not only for responding to heat stress, but also is used as a housekeeping protein, chaperoning proteins to ensure that they fold properly. Furthermore, it is known that a reduction in the activity of HSP-90 is conducive to thermotolerance (Sangster *et al.* 2008). In searching for naturally occurring HSP-90 inhibitory compounds (that might be active in cancer therapy), Turbyville *et al.* (2006) came upon a diverse community of plant–fungal symbioses. Within this population, two fungi, *C. chiversii* and *P. quadriseptata*, were isolated from several cacti, including the Christmas cactus *Opuntia leptocaulis*. *C. chiversii* and *P. quadriseptata* express related HSP-90 inhibitors, radicicol and monocillin I, respectively.

In order to demonstrate that monocillin I (MON) can enhance thermotolerance in *Arabidopsis thaliana* (a model system plant that does not normally express MON), McLellan *et al.* (2007) first confirmed that MON had cross-species affinity to *Arabidopsis* HSP-90. Once MON was shown to have affinity to *A. thaliana* HSP-90, *P. quadriseptata* was incubated within *A. thaliana* and heat shocked. This shock enhanced MON production in the fungus, which increased thermotolerance in *A. thaliana*.

In order to elucidate the pathway by which MON acts, *P. quadriseptata* was tested in *A. thaliana* HSP-101 knockouts. HSP-101 is known to be essential to heat shock response, and it is normally inhibited by HSP-90. Growing the HSP-101 mutant *P. quadriseptata* in a heat stress environment had no effect on thermotolerance, supporting the theory that *P. quadriseptata* is conferring thermotolerance by affecting the levels of HSP-101. The implications of this may be wide-ranging (McLellan *et al.* 2007). *P. quadriseptata* is not only found in cactuses, but also in maize (*Zea mays*), a species that is not phylogenetically close to *O. leptocaulis*. It may be that fungal production of HSP-90 inhibitors is a common means of thermotolerance in desert and other plants that are consistently exposed to heat stress (McLellan *et al.* 2007).

A major question is why plants have not acquired their own HSP-90 inhibitory signal for heat stress (Rodriguez & Redman 2008). A potential explanation is the energetic cost that independent production would require not only of the production of a protein, but also of a temperature-dependent regulatory pathway. Plants that provide a more hospitable environment for the fungus with an HSP-90 inhibitory compound would be more heat tolerant. The desert environment would also select for the fungus that gave the most thermotolerance to the plant. Ultimately, by selecting corresponding traits in each partner, the environment would be selecting for the holobiont itself. This reciprocal signalling between fungus and plant would not be terribly different from the tissue–tissue signalling or reciprocal induction, which is a common theme in development. There are multiple interacting levels of selection operating here (Waddington 1953; Gilbert & Epel 2009).

6. PEA APHIDS AND *BUCHNERA APHIDICOLA*

The pea aphid *Acrythosiphon pisum* and its bacterial symbiont *B. aphidicola* bacteria have become a widely accepted model for a mutually obligate symbiosis. That is, neither the aphids nor the bacteria will flourish without their partner. Pea aphids rely on *Buchnera* to provide essential amino acids that are absent from their phloem sap diet (Baumann 2005). In exchange, the pea aphids supply nutrients and an intracellular niche that permit the *Buchnera* to grow and reproduce (Sabeter Muñoz *et. al.* 2001). Because of this interdependence, aphids are highly constrained to the ecological tolerances of *Buchnera* (Dunbar *et al.* 2007). In the field, temperatures ranging from 25 to 30°C result in pea aphids with lower densities of *Buchnera* (Montllor *et al.* 2002). A recent study (Dunbar *et al.* 2007) showed that heat tolerance of pea aphids and *Buchnera* could be destroyed with a single nucleotide deletion in a heat shock gene promoter. It is important to note that secondary symbionts, such as *Serratia symbiotica*, have also been implicated in *A. pisum* response to heat shock (Russell & Moran 2006), suggesting a complex interplay of multiple genomes under thermal stress.

Although the interactions of more than two genomes may be responsible for optimal heat responses, Dunbar *et al.* (2007) have discovered that a single-base deletion in the promoter of the *Buchneria ibpA* gene can lower the thermotolerance of the aphid holobiont. This microbial gene encodes a small HSP, and the small deletion eliminates the transcriptional response of *ibpA* to heat. *Buchnera* are at least partly able to survive at such high temperatures because of constitutive expression of genes that are normally upregulated in response to heat (Wilcox *et al.* 2003), and aphids containing *Buchnera* without the single-base deletion are able to thrive under temperatures as high as 35°C in the laboratory (Dunbar *et al.* 2007).

Clones (or 'lines') with this deletion can be maintained in the laboratory, and the deletion is present in field populations, suggesting a selective advantage under certain environmental conditions (Dunbar *et al.* 2007). Although pea aphids harbouring *Buchnera* with the short *ibpA* promoter allele suffer from decreased thermotolerance, they experience increased reproductive rates under cooler temperatures (15–20°C). Aphid lines containing the short-promoter *Buchnera* produce more nymphs per day during the first 6 days of reproduction compared with aphid lines containing long-allele *Buchnera*. This trade-off between thermotolerance and fecundity allows the pea aphids and *Buchnera* to diversify. Given that pea aphids can survive with *Buchnera* in various geographical environments, the genomes need to be presumably adapted to different temperatures and climates. As Rosenberg *et al.* (2007) have pointed out, advantageous mutations will spread more quickly in bacterial genomes than in host genomes because of the rapid reproductive rates of bacteria. In an environment where heat stress is less common, a mutation that increases the reproductive rates of the host (at the cost of heat tolerance) will provide advantages to both organisms. The pea aphids and *Buchnera* both produce more progeny. The interdependence of

pea aphids and *Buchnera* suggests that the genomes of symbiotic organisms act in concert. Rather than isolated genomes, they function as a single unit. Depending on the conditions, the survival of the holobiont depends on the type of *Buchnera* inherited. In this manner, variant *Buchnera* genomes can be thought of as alleles for the larger hologenome. The inheritance of different alleles in maternally transmitted symbionts can be a critical means of providing genetic diversity within clonal populations, such as pea aphids. Just as certain alleles in a species population may be more advantageous, certain genomes may be more advantageous for the holobiont.

7. CONCLUSIONS

The three examples in this paper provide evidence that symbiosis and evolution are not separate phenomena. Evolution shapes and selects for symbiosis, while organisms in symbiotic relationships evolve to accommodate each other. Although there is tension between the needs of the individual organisms and the relationships among the symbionts, symbioses continue to exist, implying that symbiosis increases the overall fitness of the individual species involved. The evidence presented here shows that different symbiont subgroups (either clades or mutations) can be selected and effect the fitness of certain populations of holobionts (i.e. what we have traditionally considered as the large individual).

Margulis (1998) has claimed that symbiosis will be shown to be critical for the origins of variation and in the formation of new species. While the evidence presented here cannot go that far, we have tried to document several evolutionary ramifications of widespread symbiotic associations.

— Developmental symbiosis appears to be a widespread phenomenon, found throughout arthropods and vertebrates. It is not relegated to remarkable exceptions, such as lichens or squids. Codevelopment may prove to be the rule, not the exception.
— Symbionts can provide their hosts with signals for development (as when *Wolbachia* provides anti-apoptotic signals for the wasp ovary or *Bacteroides* induces gene expression in the mammalian gut) and for homeostasis (as in the heat tolerance provided by various symbionts).
— Such symbiosis can provide selectable variation. The symbioses of corals with their dinoflagellates and aphids with their bacteria indicate that genotypic variants of the symbiont can be selected by the environment.

While we have documented that symbionts can provide selectable epigenetic variation for *homeostatic* functions (i.e. thermotolerance), and we have documented cases of developmental symbioses, we have not documented cases wherein allelic or clade differences in the symbiont population affects the *development* of the host in different ways. But experiments on mice and wasps are certainly pointing in this direction. When mice with mutations in their leptin genes become obese, their guts contain a 50 per cent higher proportion of Firmicutes bacteria and a 50 per cent reduction in Bacteroidetes bacteria than wild-type mouse guts. Moreover, when the gut symbionts from the obese mice were transplanted into genetically wild-type germ-free mice, these mice gained 20 per cent more weight than those germ-free mice receiving gut microbes from wild-type mice (Ley *et al.* 2005; Turnbaugh *et al.* 2006). Thus, there may be an interaction between the genotype of the host and the types of microbial symbionts that are selected by that host environment. Together, they generate a particular phenotype, in this case, obesity. Similarly, studies are beginning to look at the relationship between the genotype of the *Asobara* wasps and the *Wolbachia* symbionts that permit oogenesis. Dedeine *et al.* (2005) have reported that different genotypes of *Asobara* interact with *Wolbachia* in different ways.

The webs of life are predicated on symbioses between plants and their rhizobacterial, endophytic and mycorrhizal symbionts. As developmental biologists begin appreciating how important symbionts are for animal development, evolutionary developmental biologists may find that some of the most important principles of evolution are in the interactions of insects and their *Wolbachia*, termites and their protists, and vertebrates whose guts teem with a consortium of microbes.

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ENDNOTE

[1]Heterochrony, heterotopy and heterometry can be described at any level of organization. Here, they will be described at the level of gene expression.

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