which was formerly thought to be one of the earliest regulators of maturation [16–18]. Specifically, they propose that DYN-1 binds to Vps34, the class III phosphatidylinositol 3-kinase that generates phosphatidylinositol-3-phosphate, and that the kinase in turn associates with Rab5, forming a tripartite complex (Figure 1). In support of their model, the authors documented the ability of these proteins to interact *in vitro* and were able to co-immunoprecipitate all three components. Curiously, Rab5 was present in the complex in its GDP-loaded, inactive state. Activation of Rab5, i.e. the conversion to its GTP-loaded form, is required for progression of maturation and the exchange of nucleotides does not occur spontaneously but requires the participation of a guanine nucleotide-exchange factor (GEF). In the case of phagosome maturation, the nature of the particular exchange factor engaged remains undefined, because the known GEFs tested by Kinchen *et al.* [1], namely RME-6, RIN-1 and Rabex-5, had no discernible effect.

The realization that Vps34 plays a role in the recruitment of Rab5 reveals a complex, reciprocal interaction between these proteins. Active Rab5 is in turn known to stimulate the catalytic activity of the kinase [19] (Figure 1). Moreover, by a mechanism that is poorly understood, the products of the activity of Vps34 serve to terminate the activation of Rab5, as revealed following treatment with the Vps34 inhibitor wortmannin, which results in a prolonged residence of Rab5 in phagosomes [20]. When taken together, these observations suggest the following sequence of events (Figure 1): firstly, dynamin recruits to the phagosome Vps34, which in turn brings inactive, GDP-bound Rab5 to the phagosomal membrane; secondly, an unknown GEF catalyzes the exchange of GDP for GTP, activating Rab5 on early phagosomes; thirdly, activated Rab5 stimulates Vps34, producing phosphatidylinositol-3-phosphate; and finally, phosphatidylinositol-3-phosphate promotes the inactivation and dissociation of Rab5 from phagosomes, probably resulting in the concomitant inactivation of Vps34 and possibly also in the dissociation of dynamin from the phagosome. This interesting model remains to be fully validated. In addition, the mechanism whereby dynamin is attracted to phagosomes, the nature of the GEF that activates Rab5 and the events connecting phosphatidylinositol-3-phosphate with the termination of Rab5 activity must be identified. Clearly, the work of Kinchen *et al.* [1] has added many interesting facets to the complicated mechanism of phagosome maturation but, in the process, has raised at least as many questions as it has answered.

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Microbial Evolution: Stalking the Wild Bacterial Species

A recent report suggests that, when habitats are disturbed, bacterial populations that would be considered to be separate species can merge, reversing the process of speciation. But, for bacteria, 'species' remains undefined and undefinable.

W. Ford Doolittle

Bacteriologists are bemused by the word 'species' and how it might usefully and truthfully be applied to their organisms of choice. As genomic and metagenomic efforts turn increasingly to complex natural

environments, the problems of defining and recognizing bacterial species in the wild and of understanding the genomic and ecological processes that might create and maintain them seem ever more urgent. Not surprisingly, papers addressing these issues directly or indirectly appear on almost a weekly basis now. In a recent report, Sheppard *et al.* [1] provide evidence for merger of two bacterial populations that should by any currently accepted rules of thumb comprise two separate species. The data are compelling, although the authors' explanation for this merger is only one of many possible, and still begs the question "what *are* species?"

An increasingly popular way of thinking about bacterial species treats their populations as if they resemble the animal assemblages for which Ernst Mayr [2] formulated his familiar 'Biological Species Concept', and such thinking clearly informs the work of Sheppard *et al.* [1]. According to this concept, species are "groups of interbreeding natural populations that are reproductively isolated from other such groups". Of course, bacteria don't actually breed — don't *reproduce* sexually — but often they do avidly engage in homologous recombination with DNA acquired from other cells of the same or different populations *via* conjugation, transduction or transformation. Indeed, in some groups, homologous recombination far outdoes mutation as a generator of genomic diversity and likely source of evolutionary novelty.

If within-population homologous recombination is sufficiently frequent and between-population homologous recombination sufficiently rare, gene sequences will stay relatively homogenous within populations — because of frequently exchanging and replacing each other — while diverging between populations that have become separated by some sort of initial physical, ecological or genetic barrier. (Genetic barriers could arise through changes in restriction/modification systems or the recognition properties of conjugation machinery, plasmids or phages, for instance). By analogy with animals, we could call such diverging populations 'species'. We could also predict that as their genes diverge further in sequence, gene flow will be further reduced, because homologous recombination requires considerable sequence similarity between the recombining DNA molecules.

Fraser *et al.* [3] have recently modeled this later process and conclude that some sort of initial barriers may indeed be generally needed to set it in train. That is, although "it is plausible that chance variation would occasionally result in strains different enough from the founder population that they no longer recombine", such accidental sympatric speciation is not likely, given known correlations between recombination rate and sequence divergence. Conversely, should the barriers be removed before sequence divergence has progressed too far, incipient species can again merge into more homogenous populations — they can 'despeciate'.

This is precisely what Sheppard *et al.* [1] think has happened among certain strains of the human (and animal) pathogen *Campylobacter*, and provides much of the interest in their report. They characterize several populations of *C. jejuni* and *C. coli* (common causes of gastroenteritis) using the popular multi locus sequence typing (MLST) approach. The technique entailed in this case the polymerase chain reaction (PCR) amplification and sequencing of fragments (alleles) of seven unlinked housekeeping genes from thousands of isolates comprising 2,953 distinct allele combinations (sequence types) each representing a sequence haplotype of 3309 base pairs. Haplotypes very largely clustered into the two designated species, although there were some interspecies hybrids showing mixed haplotypes, the result of homologous recombination. More tellingly, such hybrids occurred predominantly within one of the three subclades (clade 1) into which the *C. coli* sequence types can be clustered, based on their analyses. Hybridity seems to result from the import of *C. jejuni* alleles, occurring at multiple occasions and at each of the seven loci, at some time after the divergence of clade 1 from clades 2 and 3 — and possibly quite recently, because many of the imports are identical in sequence to alleles in the donor population. The authors predict that "if maintained over time, these rates would lead to progressive genetic convergence" unless this were in some unlikely way prevented by selection.

Thus, two distinct and well-recognized species (*C. jejuni* and *C. coli* show only 86.5% sequence identity among housekeeping genes, well below the ~94% that characterizes most recognized species [4]) are becoming again one — they are despeciating. Sheppard *et al.* [1] find that the imported *C. jejuni* alleles are more like those found in domesticated (ruminant and poultry) than in wild bird hosts, and speculate that changes in agricultural practices are responsible for this reversal of what we might take as the more usual divergent evolutionary course. But surely other causes, such as the availability of transducing phages that infect *C. jejuni* and *C. coli* clade 1 more effectively than clades 2 and 3, would also do the trick.

Sheppard *et al.* [1] suggest that this work "provides an opportunity to observe evolution by hybridization as it is occurring" and that with further studies of this sort "we can hope to mitigate some of the harmful consequences of both environmental change and the biotic response to it". Perhaps in the very long term — if we have that luxury — this will come true. But more immediately we can ask what this paper can tell us about 'speciation', 'despeciation' and 'species', words that appear together more than thirty times in its text. Surely it is because they purport to address the reality, nature and importance of the biological entities and processes to which these terms refer that papers such as this one of Sheppard *et al.* [1] (and indeed some of ours [5]) attract broader interest.

Certainly, *C. jejuni* and *C. coli* clade 1 are not behaving like good species here: the barriers between them are too leaky. There also is allelic exchange between the three subclades of *C. coli*, and no reason to believe that tightness of the clustering that is observed with MLST would not relax further if the environment were sampled more broadly. Nothing is unexpected about this: surely there must be in Nature a broad spectrum of rates of inter-individual, inter-genome exchange determined by a broad spectrum of geographic and ecological restrictions of differing negative strengths, and agents or facilitators of exchange (conjugation machinery, phage specificity, mutations of the mismatch repair

system) with qualitatively and quantitatively different and graduated positive effects. Although sometimes these may conspire to produce groups with a degree of within-population cohesion and between-population divergence that would have satisfied Ernst Mayr, there is no reason that this always or even often has to be so.

Much of the literature proceeds from the notion that species are real — if for no other reason than that systematists and ecologists seem to need them [6] — and will take any level of clustering as evidence. But no reasonable population model would have the data lack all structure. So unless there is some general agreement in advance about what degree of clustering Mayr (or we) would require, there is no principled way to decide whether papers like that of Sheppard *et al.* [1] really speak to us about 'species', 'speciation' and 'despeciation'.

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Chordate Metamorphosis: Ancient Control by Iodothyronines

A new study shows that iodothyronines induce metamorphosis in the cephalochordate amphioxus by binding to a receptor homologous to vertebrate thyroid hormone receptors. Iodothyronine-induced metamorphosis may be an ancestral feature of the chordates.

Robert J. Denver

A complex life cycle, where an animal begins life as a larva then undergoes a metamorphosis to the juvenile adult form, is a widespread and ancient life history strategy [1]. Larvae generally exploit different ecological niches from adults, thus avoiding competition for resources. There is considerable morphological diversity among larvae and the transformations that they undergo during metamorphosis [1], which raises the question whether the complex life cycles of extant species reflect an ancestral or a derived state.

Among chordates with complex life cycles, the best studied are the anuran amphibians (frogs and toads). Anuran larvae (tadpoles) are aquatic, and undergo morphological, biochemical and physiological transformation into the terrestrial juvenile adult. Gudernatch [2] first showed that vertebrate thyroid glands contain an active component that induces precocious metamorphosis when fed to tadpoles. Thyroid hormone is now known to orchestrate the diverse morphological and physiological changes that occur during amphibian metamorphosis [3]. Thyroid hormone has also been shown to control flatfish metamorphosis [4], and exogenous thyroid hormone can induce metamorphosis in echinoderm larvae [5–7]. Now Paris *et al.* [8] have reported in *Current Biology* that metamorphosis of the cephalochordate amphioxus can be induced by iodothyronines.

The active component in vertebrate thyroid glands is 3,5,3′5′-tetraiodothyronine (thyroxine; T_4) , a member of a class of compounds known as iodothyronines, which are derived from two tyrosine residues of a precursor protein which have iodine atoms attached to the aromatic rings [3]. Thyroxine is generally considered to be a secondary precursor that must be converted to the biologically active form of the hormone, 3,5,3′-triiodothyronine (T_3) [9]. The actions of thyroid hormone are mediated by thyroid hormone receptors, which are ligand-activated transcription factors belonging to the steroid hormone receptor superfamily [10]. All jawed vertebrates that have been studied possess two thyroid hormone receptors, TRα and TRβ, which bind to DNA as dimers, with the preferred configuration being a heterodimer with retinoid X receptor (RXR) [11]. The thyroid hormone receptors have been shown to be essential for metamorphosis of the clawed toad *Xenopus laevis* [12].

Thyroid hormone controls metamorphosis in vertebrate species, but the evolutionary origin of this developmental signaling in chordates is unknown. Most extant urochordates and cephalocordates have a complex life cycle [13]. The cephalochordate amphioxus, now considered to be among the most basal members of the phylum Chordata [14], have larvae that are asymmetric, with the mouth on the left side, and gill slits on the right side of the body (Figure 1; reviewed in [13]). At metamorphosis the pelagic larva transforms into a benthic adult. The mouth moves medially from its left lateral position, and the primary gill slits move from right to left. A secondary set of gill slits develop simultaneously on the right side of the animal. Iodothyronines are produced by the endostyle of amphioxus, a structure considered the precursor of vertebrate thyroid follicles [13,15]. Over 40 years ago, it was hypothesized that amphioxus metamorphosis is controlled by iodothyronines; however, only one attempt was hitherto made to address this hypothesis, with results complicated by an incomplete experimental design [16].

In their new work, Paris *et al.* [8] found that precocious metamorphosis in larval amphioxus is induced by treating with T_3 or T_4, although T_3 was