

# The Evolution of Multispecies Populations: A Multilevel Selection Perspective

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## Abstract

Two or more independent species lineages can fuse through an evolutionary transition to form a single lineage, such as in the case of eukaryotic cells, lichens, and coral. The fusion of two or more independent lineages requires intermediary steps of increasing selective interdependence between these lineages. We argue a precursory selective regime of such a transition can be Multilevel Selection 1 (MLS1), as intraspecies MLS1 can be extended to ecological multispecies arrangements. To establish this we develop a trait group selection (MLS1) model applicable to multispecies mutualistic interactions. We then explore conditions under which such a model could apply to mutualistic relationships between pollinators and plants. We propose that MLS1 could drive transitions towards higher interdependency between mutualists and stabilise obligate mutualisms in the face of invasion by cheater variants. This represents a radical extension of multilevel selection theory, applying it to the evolution of multispecies populations, and indicating new avenues for researching ecological community evolution.

# 1. Introduction

The evolutionary models that emerged from the modern synthesis often assume a conspecific population with no spatial structure and with random mating (e.g., a classic Wright-Fisher population, Fisher 1930, Wright 1931). Such models are well suited to explain complex traits that evolved in a stepwise fashion when each change in phenotype is assumed to have been accompanied by an incremental increase in fitness. But they are not sufficient to explain traits that defy the “prime directive” of evolution, which in theory is the maximization of individual fitness (e.g., *The Selfish Gene*, Dawkins 1976).

Such is the case for the evolution of altruism, where an individual whose behavior increases the fitness of conspecifics within the same population suffers a reduction in fitness for their efforts. Models that explain the evolution of altruism, and of cooperation<sup>1</sup> in general (see West et al. 2007), often require more than what is assumed for a Wright-Fisher population. Kin selection theory (Hamilton 1964, Frank 2013), for example, appeals to features intrinsic to individuals, such as the ability to recognize kin or the limited dispersal of offspring. Multilevel selection theory (Damuth & Heisler 1988, Okasha 2006) makes use of the spatial structure of a population (i.e., a metapopulation) combined with a process of dispersal whereby groups episodically exchange individuals; these features can be construed as ecological insofar as they are imposed by the environment (e.g., Black et al. 2020). The salient point that unifies the two theories is that individual and ecological features can give rise to what has been called *population structured selection* (Kerr & Godfrey-Smith 2002), where selection occurs by virtue of the non-random spatial structure and/or patterns of dispersal and/or patterns of interaction between individuals.

Population structured selection favors altruism when it causes the benefit of the altruistic behavior to be preferentially conferred to other altruists (Fletcher & Doebeli 2006, 2009). Two scenarios are considered under the ambit of multilevel selection (MLS) theory<sup>2</sup>. In the first (MLS1, Damuth & Heisler

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<sup>1</sup> Cooperation is the broad category of behaviors that can be fixed by positive selection because of their beneficial effect on recipient individuals, where cost and benefit are measured in terms of the expected number of offspring generated over the lifetime of the individual. Altruism (altruistic cooperation), a behavior that is costly to the actor but beneficial to the recipient, can be fixed by positive selection when the actor gains indirect fitness benefits that outweigh the cost. Mutualism (mutually beneficial cooperation) occurs between species when the behavior of each provides a benefit to the other regardless of whether those behaviors are costly (see West et al. 2007 for a compendium of terms associated with cooperative behavior).

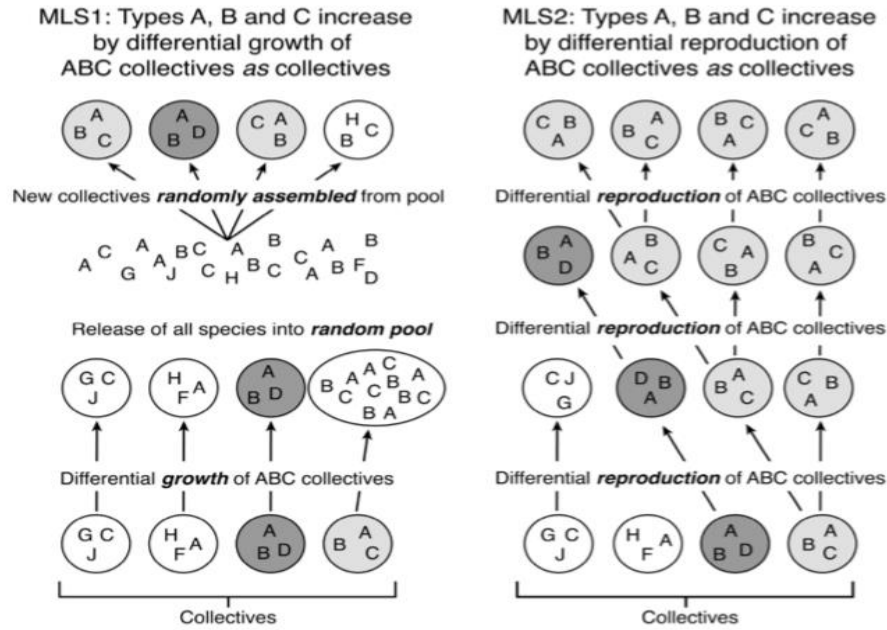
<sup>2</sup> It has been argued that MLS1 and MLS2 are not the distinct categories represented in Multilevel Selection Theory (Okasha 2016; Bourrat 2021). We do not have a strong commitment to the MLS1/MLS2 distinction being always well-defined but utilise it as it is the primary framework for describing transitions in the levels of organisation, and the MLS1/MLS2 is a good shorthand description of the community dynamics that we wish to discuss. Alternative frameworks, which could be similarly used are the “Staying together” and “Coming together” framework of Tarnita et al. (2013).

1988), the fitness of an individual is assumed to be the sum of a component attributed to its character state (genotype or phenotype) plus a contextual component that is a function of the mean character state of all individuals within the group in which it exists. The classical scenario occurs when the mean fitness of a group is correlated with the proportion of altruists it contains. In the second (MLS2, Damuth & Heisler 1988), the group itself is assigned a fitness that reflects its ability to reproduce as a group. Groups are therefore equated to Darwinian individuals and the collection of groups to a Darwinian population (Godfrey-Smith 2009). Population structured selection by other means, such as one of the mechanisms considered under kin selection theory (e.g., kin recognition), is also possible. Indeed, kin selection and multilevel selection theories have much in common, differing mostly in their mathematical details (Queller 1992, Bijma & Wade 2008, Fletcher & Doebeli 2009, Birch & Okasha 2015).

The most well-known model of MLS1 is trait-group selection (Wilson 1975). Here dispersal occurs episodically (e.g., once every generation) when all individuals in the metapopulation are gathered into a common pool and then redistributed to form new groups. This process mimics the life-history of species in which “individuals are spatially restricted during most of their life cycle, with the exception of their dispersal phase” (ibid). Trait-group selection can result in the fixation of altruism<sup>3</sup> by giving genotypes associated with larger groups (altruists) a chance to displace genotypes associated with smaller groups (non-altruists), despite the individual-level fitness deficit suffered by altruists. However, there are other ways that MLS1 can arise that incorporate different patterns of group fissure and fusion while retaining a focus on lower-level, or particle, fitness within the context of a group (e.g., Okasha 2006 pp 51, Goodnight 2011). The most common way to force groups to evolve by MLS2 is to cull groups weak in each group-level trait and then form new groups by splitting those that remain. In this way, group-level traits can be reliably transmitted to offspring groups, and groups can exhibit the key features of Darwinian individuals, namely variation, differential reproduction, and inheritance (Lewontin 1970). MLS1 and MLS2 are illustrated in Fig. 1.

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<sup>3</sup> Altruism is said to be strong under the scenario where a non-altruistic individual, if it were to convert to an altruist, would suffer a loss in fitness; altruism is otherwise said to be weak (Kerr et al. 2004, Okasha 2006). Trait-group selection can only support the evolution of weak altruism because episodic pooling and redistribution homogenizes the composition of groups (Wilson 1980, Okasha 2006 pp 192-197).



**Figure 1.** On the left is a depiction of a trait group selection model of multilevel selection 1 with differential selection of individuals in groups going into a common pool and then reassembling into groups. On the right is multilevel selection 2 with reproduction and selection of groups.

In what follows we investigate the conditions under which multispecies ecological arrangements (Wilson 1975; Wade 1978) might evolve via population structured selection by extending MLS theory from conspecific to heterospecific populations. Here altruism is replaced by interspecies cooperation or mutualism. Although scenarios with mutualistic interactions that are consistent with MLS2 exist in nature (e.g., arguably in phoretic associations, Wilson & Sober 1989), we argue that this is uncommon. A more likely scenario is the piecemeal migration of small groups of conspecifics that either fill vacant ecological niches or invade occupied territory to displace resident groups. We posit that community composition (i.e., the relative frequency of the various genotypes of the various species) associated with greater productivity might be re-produced<sup>4</sup>, in a manner of speaking, via a gradual assembly process. Communities of mutualists engaged in interspecies cooperation are expected to be more productive than communities of non-mutualists. This gives the constituent populations of communities of mutualists an advantage in numbers which, in combination with an MLS1 dispersal process, can lead to the reassembly of the same community structure in other ecological patches.

<sup>4</sup> Doolittle & Inkpen (2018) make a biologically informed distinction between scenarios of reproduction, where there is a direct association between parent and offspring, and scenarios of “re-production”, where entities such as communities of microbes have no such association but can nevertheless be “created again”.

Whereas previous researchers have suggested that multispecies ecological arrangements can be subject to selective processes (Wilson 1997; Swenson 2000; Whitham et al. 2020) there has been little discussion of MLS1 in this context. A notable exception is Frank’s (1994) creation of a kin selection model of a spatially correlated mutualistic pairing, but there has been limited further study of this model apart from some simulations of the dynamics (Yamamura et al., 2004; see Akçay 2015). Our objective is to defend the possibility that multispecies MLS1 is plausible in nature and that, when it occurs, it can lead to the evolution of mutualistic interactions between the constituent populations of a community. Given fitness in MLS1 is assessed on the lower-level, we believe MLS1 is amenable to multispecies populations as both species will be evolving due to their own unique species specific selective regimes in addition to their group context. Our particular focus is on whether the ubiquitous mutualistic relationships that occur throughout natural systems might be explained by some form of multispecies MLS1 in a metacommunity setting via the assembly process alluded to in the previous paragraph.

To this end, we first introduce some of the current work on community-level selection, contending that too often the focus has been on MLS2 (S.2). Next, we suggest how MLS1-like processes in a metacommunity setting might lead to mutualism, in contrast to the prevailing explanation of coevolution within a multispecies community (S.3). We use a simple model of multilevel multispecies selection to investigate the worst-case scenario where mutualistic behaviours are costly to the actor. We use this model to show (1) that mutualism is unlikely to arise in this scenario by coevolution alone; and (2) how it might arise under MLS1 by the “amplification of genetic drift” when population size is limited by the ecological milieu (S.4). Finally, we present a few real examples of multispecies ecological arrangements and discuss how they might be explained by our modelled process (S.5).

## **2. Multispecies Arrangements and MLS2**

A foundational principle of biology is that natural selection increases the frequency of the genotype that produces the most offspring over the next generation regardless of the long-term viability of the population or group. Yet this bromide only applies to unstructured populations. Population structured selection by MLS1 can reward genotypes with lower fitness if the reduction in fitness also improves the productivity of the group (i.e., the rate at which the group increases in size or the size of the group at birth-death equilibrium). A paradigmatic example is the evolution of whole-group trait altruism among populations of microbes, where altruistic individuals incur a reduction in fitness by producing a public good

(e.g., iron-scavenging siderophores) that benefits all members of the group, including itself (Griffin et al. 2004, West et al. 2006, Niehus et al. 2017).

Transitions of independent species becoming multispecies evolving populations undoubtedly occur as evidenced by the existence of symbioses in nature (eukaryotic cells, lichens, etc.). We should therefore expect to see natural communities with properties somewhere *between* those composed of independent individuals reproducing in populations and populations who reproduce as a multispecies unit. Take, for example, the multispecies assemblages of mites, nematodes, fungi, and microbiota that exist in phoretic communities built around an insect host. In their focal example, Wilson & Sober (1989) show how a certain beetle (*Scolytidae*) that burrows into a tree relies on the fungi (*Ceratocystis*) it carries to stop the tree from sealing the burrow with sap. They contend that “It is not an exaggeration to call such a functionally organized community a multi-species superorganism” (p. 349), implying an MLS2-like process of reproduction. In this case, the community phenotype is the ability to exploit the tree's resources, which benefits most if not all constituent populations. Community reproduction of a kind occurs when offspring beetles assemble their microbiota from their parents and the local environment.

Construing the differential “re-production” of communities as if it were community-level selection is not uncommon, particularly in the literature on microbial communities and the biofilms they produce (Ereshefsky & Pedroso 2013). Some authors go so far as to say that the process of resource recycling associated with some communities (i.e., syntrophy) can be viewed not only as a community-level phenotype subject to selection but also as a unit of selection itself (Doolittle & Booth 2017, Doolittle & Inkpen 2018). Similarly controversial is the claim that communities formed by the joining of a microbial assemblage with its host organism, the so-called “holobiont”, constitutes a multispecies individual (Zilber-Rosenberg & Rosenberg 2008; Roughgarden et al. 2018). The problem with such cases is that, by equating the reassembly of communities to community-level reproduction, they treat the whole community as the bearer of fitness and implicitly assume MLS2. The assumption that ecological communities reproduce as if they were units of selection holds them to a higher standard than is necessary to explain the existence of interspecies cooperation.

Experimental studies of community selection have been nearly entirely based on processes that give rise to MLS2, namely the culling of communities weak in the property selected by the researcher and the recolonization of empty patches by propagules drawn from the surviving communities that are strong in the researcher selected property (that is, culling-and-recolonization). Examples include the empirical study of Swenson et al. (2000), where artificial selection was imposed onto communities of microbes consisting of thousands of species, as well as the *in silico* studies of Williams & Lenton (2007) and Doucier et al. (2020). These studies artificially create MLS2 by imposing a reproduction-like process onto

communities (Lean et al. 2022). Natural ecological arrangements are highly unlikely to result in MLS2 as such group selection involves the “re-production” of a collective that comprises multiple species (Lean et al. 2022). Reproduction involves ‘vertical transmission’, or inheritance occurring from a parent to offspring.

Of course, cases of MLS2-like multispecies reproduction can be found in nature. Aphids directly transmit their endosymbionts, the *Buchnera* bacteria, to their progeny, which the next generation of aphids requires to survive. However, the focal species in most ecological arrangements do not inherit interacting populations from their parents. Instead, they gain them from the environment. This is sometimes described as “horizontal inheritance”, but it is more aptly described as “horizontal assembly”. The point is that communities comprised of horizontally assembled individuals are not distinct units of selection because they lack the necessary parent-offspring resemblances associated with vertical inheritance. And because such communities do not reproduce as communities, they cannot be construed as units of selection (Maclaurin & Sterelny 2008, Lean 2020, c.f. Bouchard 2014, c.f. Dussault & Bouchard 2017). This is the main objection to the notion that a holobiont is a unit of selection (Booth 2014; Skillings 2016, Douglas & Werren 2016). Note, however, that replacing vertical reproduction with horizontal assembly does not preclude population structured selection. Indeed, something like multispecies MLS1 may well explain the prevalence and spread of coordinated adaptations throughout ecosystems, as we will argue.

### 3. The Plausibility of Multispecies MLS1

There are strong a priori reasons to suspect that many multispecies arrangements undergo MLS1 selective dynamics in nature. The first is based on models of “transitions in the levels of organisation” (Szathmáry & Maynard Smith 1995, Michod 2011, Rainey & Kerr 2011). The formation of lichens, endosymbionts, coral, and other cases where two populations fuse their reproduction and form aggregated units of selection show that multispecies populations can transition to an MLS2-like mode of reproduction. Models of such transitions typically assume that a system *must* undergo some form of population-structured selection before attaining the new level of organization, or that there must first be MLS1 for there to be an MLS2 produced entity<sup>5</sup> (e.g., Okasha 2006). While it is unclear what proportion of natural populations have

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<sup>5</sup> A counterpoint to this is when antagonistic co-evolution can foster the fusion of two lineages into a MLS2 entity. Parasites can become so entwined in their host that they are then vertically rather than horizontally transmitted. When the two genotypes are reproduced together their high mutual dependency fosters mutualism creating symbioses from parasitism (Yamamura 1993, Nalepa 2020). In this case the negative effect of the parasite population suppresses the

ever achieved such a transition, it is likely to be quite small due in large part to the difficulty in establishing coordinated reproduction between organisms with different life histories. It can therefore be argued that, because we know that two species can transition to an MLS2-like form of reproduction and that such an outcome is uncommon, and because we assume that something like MLS1 is required to achieve it, there is good reason to suspect that more multispecies assemblages have undergone some form of MLS1 in nature than those that have reached MLS2<sup>6</sup>.

A second reason to suspect that multispecies MLS1 may be common involves the problem of cheating. Suppose species *A* and species *B* engage in reciprocal mutualism, each conferring a benefit to the other. Further, suppose that conferring a benefit comes with a slight reduction in fitness. An arrangement of this type will be stable in a single community provided the benefit of mutualism for each species is greater than its cost (cf. Hamilton 1964). Suppose, however, that this is not the case and that there are cheaters, say *B'*, that can exploit the benefit provided by species *A* without providing anything in return. The cheater *B'* does not pay the cost of the mutualism. It may therefore displace the mutualist *B* population by positive selection. The mutualism will therefore always be vulnerable to cheaters in the absence of some mechanism of defence. One such mechanism is the ability of each species to recognize its mutualistic counterpart so that it can refrain from interacting with cheaters. In this case, defence is based on an individual-level trait that may conceivably arise by coevolutionary processes within a single community. Note, however, that this would require macroevolutionary time scales as the system awaits the appropriate individual-level traits to arise. Cheaters, if they arise spontaneously in the population, might therefore displace their mutualistic counterparts before such an event occurs.

An alternative mechanism is MLS1 within a metacommunity. MLS1 provides a defence against cheaters whenever larger populations, assumed to be those composed of a higher proportion of mutualists, are given the opportunity to overwhelm smaller populations by sheer weight of numbers. Any dispersal process whereby individuals are episodically exchanged between communities (by migration, e.g., Wade 2016, or trait-group selection, Wilson 1975), can stabilize mutualistic interactions over ecological time scales by displacing communities with a higher proportion of cheater variants. This explanation has the advantage that it does not require individual-level traits to sanction cheaters. Instead, sanctions are *imposed* onto the community by the ecological conditions presumed to have given rise to MLS1 (i.e., by an

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fitness of the host species meaning that it does not promote both species. So, MLS2 can form without an intermediate stage of MLS1. Thanks to Ford Doolittle for this point.

<sup>6</sup> This does not necessitate that there are more cases currently of MLS1 populations than MLS2. All adults develop from children but there are not necessarily more children in the world. If there was a brief transitional period from individual to MLS2 via MLS1 and MLS2 is an evolutionarily stable configuration we would find more MLS2 than MLS1. Thanks to Ford Doolittle for this point.



“ecological scaffold”, Black et al. 2020). Furthermore, MLS1 can give mutualists time to coevolve individual-level traits that sanction cheaters, potentially removing the need for the conditions that gave rise to MLS1 in the first place (cf. Bourrat 2021). The *origin* of mutualistic interactions might therefore be more plausible under some form of MLS1, whereas its subsequent *maintenance* (under conditions where novel cheater variants do not invade) may be more aptly explained by coevolutionary processes<sup>7</sup>.

To provide an example, consider the obligate mutualism between the long-proboscis fly, *Moegistorhynchus longirostris* and the long-tubed irise, *Lapeirousia anceps* (Pauw et al. 2009). These species are highly adapted to each other by virtue of their peculiar phenotypes to such an extent that invasion by cheaters is unlikely. The iris requires the fly because other species cannot access its pollen and the fly’s unwieldy proboscis makes it ill-adapted to exploit the resources of other flowering plants. Such a relationship may have started with a patchy environment in which groups of an ancestral flower (species *A*) were spatially separated (forming a metapopulation) and where there was a wind-driven dispersal process that carried seeds from one patch to another (imposing migration). At the same time, there may have been an ancestral pollinator (species *B*) that dispersed by seasonal pooling and redistribution (trait-group selection). The ancestral flower may have been pollinated by multiple species, and the ancestral pollinator may have availed itself to resources proffered by other flowers. However, assuming mutualism increases productivity, a facultative mutualism between species *A* and *B* may have been fixed in their respective populations via a process of multispecies MLS1. Once fixed, it would then have been possible for the two species to coevolve, either by neutral processes or under some other selective force, to become increasingly specialized to one another. In this way, we argue, multispecies MLS1 can play a role in maintaining obligate mutualisms when novel cheaters invade. This claim is explored using a simple theoretical model in the next section.

Given the plausibility of MLS1 as an intermediary, why has it not been extensively studied? The source of this is the preference to ascribe multispecies evolutionary interactions as co-evolution. Co-evolution is the reciprocal evolutionary change in interacting species. Studies into coevolution have been persistent in ecology and evolution since the 1960s (e.g., Ehrlich & Raven 1964). In Pauw et al. (2009),

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<sup>7</sup> This weight of numbers mechanism for mutualistic co-ordination could allow for the evolution of correlated interactions between mutualistic partner species and be combined with them in future models. For example, Forster and Wenseleers (2006) consider the role of the following mechanisms in mutualism evolution: co-operator association, partner-fidelity feedback, and partner choice (also see Akçay 2015). The mechanisms act to maintain the correlated interaction that allow for mutualistic adaptations to persist within a community. These could also be a significant means by which community reassemble may be biased towards the creation of mutualistic associations. When weight of numbers is combined with biased community reassemble the correlation between mutualistic interactions would be significantly strengthened.

which discusses the relationship between long-proboscis flies and long-tubes irises, the word “coevolution” appears 48 times, but “multilevel selection” is never mentioned. The source of the preference to ascribe these interactions to co-evolution may be the emphasis on parsimony (i.e., Williams 1966). Coevolution at a single level of organization (i.e., within a single community) is *prima facie* more parsimonious than MLS1 with its need for spatially hierarchical population structure and/or a process of dispersal. Given that it is empirically difficult to differentiate MLS1 from co-evolution, co-evolution has been the preferred explanation even though MLS1 may be a more apt model of these evolutionary relationships in some cases.

## 4. A Simple Model of Multispecies MLS1

Here we present a simple mathematical model and verbal argument<sup>8</sup> to demonstrate the application of MLS1 to multispecies populations. We assume two species, one a flower (species *A*) and the other a pollinator (species *B*). Each is assumed to have two variants, one that is cooperative (*C*) and one that is selfish (*S*). It is assumed that a cooperative variant of one species confers a fitness advantage to both variants of the other species at a cost to its fitness. Hence, a cooperative flower might produce more nectar, which benefits both the cooperative and selfish pollinator, but at a cost in the form of a reduction in its ability to produce pollen. Likewise, a cooperative pollinator might have features that enhance its ability to gather and convey pollen, which benefits both the cooperative and selfish flower, but at a metabolic cost that reduces its ability to produce offspring. In this way, we set up a trade-off scenario where the maximization of individual fitness is in opposition to the formation of mutualistic communities consisting of cooperative types alone.

### 4.1 A Simple Single Community Model

Table 1: Hypothetical Payoff for Pairwise Interspecies Interactions

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<sup>8</sup> In this article we only aim to present the hypothesis that multispecies MLS1 can explain the evolution of some forms of interspecies cooperation. Testing the hypothesis via computer simulation would be the natural next step. However, such tests are technically complex and beyond the scope of our current objective.

	<b>Selfish Pollinator (B)</b>	<b>Cooperative Pollinator (B)</b>
<b>Selfish Flower (A)</b>	$(f_A, f_B)$	$(f_A + b_A, f_B - d_B)$
<b>Cooperative Flower (A)</b>	$(f_A - d_A, f_B + b_B)$	$(f_A + b_A - d_A, f_B + b_B - d_B)$

Let  $f_A$  represent the baseline fitness assigned to flowers in the absence of interactions with either pollinator variant (Table 1). Flowers and pollinators are assumed to interact randomly, so the fitness of each flower variant is a function of the proportion  $\pi_B^C$  of pollinators that are cooperative. The selfish flower gains a benefit  $b_A$  when it interacts with (or “cheats”) a cooperative pollinator. Its fitness is therefore:  $w_A^S = f_A + \pi_B^C b_A$ , where  $b_A$  (“ $b$ ” for benefit) is the benefit that the cooperative pollinator confers to both kinds of flowers. The fitness of a cooperative flower is similarly expressed, but also accounts for the cost  $d_A$  (“ $d$ ” for deficit) of the cooperative behaviour:  $w_A^C = f_A - d_A + \pi_B^C b_A$ . The expressions for the fitness of the selfish and cooperative pollinator are similarly constructed:  $w_B^S = f_B + \pi_A^C b_B$  and  $w_B^C = f_B - d_B + \pi_A^C b_B$ .

It will be assumed that each variant, selfish or cooperative, produces offspring that are likewise selfish or cooperative and that there is no mutation or drift. This facilitates the use of a simple expression for the deterministic change in the proportion of flowers or pollinators that are cooperative over one generation using the Price equation (Price 1970, Okasha 2006, Frank 2012):

$$\Delta\pi_A^C = \pi_A^C \left( \frac{w_A^C}{\bar{w}_A} - 1 \right), \Delta\pi_B^C = \pi_B^C \left( \frac{w_B^C}{\bar{w}_B} - 1 \right) \quad (\text{eq. 1})$$

It can readily be seen from eq. 1 that the proportion of cooperative variants of both types will increase over the next generation if and only if their fitness is greater than the average fitness of their respective populations, i.e., iff  $w_A^C > \bar{w}_A$  and  $w_B^C > \bar{w}_B$ . The point of this simple exercise is to show that this is never the case in a population containing both cooperative and selfish variants. Hence, unless we assume an individual-level trait that permits cooperative pollinators to preferentially interact with cooperative flowers, the selfish type of each species is expected to be fixed, and the cooperative type to be eliminated, within a single community. It follows that the flower-pollinator mutualism, as we have modelled it, cannot arise within a single community by selection.

## 4.2 MLS1 in a Metacommunity

It is important to note that eq. 1 does not account for stochastic processes, which include genetic drift. If we assume a metacommunity (for the moment, without dispersal) composed of many small flower-pollinator communities, it will be possible for the cooperative variant of either species to reach fixation by drift. That is, within any single community composed of selfish types alone (a selfish community), cooperative flower or pollinator mutants might occasionally arise and be fixed by random processes to form a community composed of cooperators alone (a cooperative community). Such a community will be vulnerable to selfish mutants, however, which can be fixed by selection due to their greater fitness.

Let  $P_{SC}$  be the probability that any given selfish community evolves by mutation-drift into a cooperative community. And let  $P_{CS}$  be the probability that a cooperative community evolves by mutation-selection back to the selfish state. Assuming the rate at which mutations arise that change a selfish type to a cooperative type and vice versa is symmetrical, it must be the case that  $P_{CS} > P_{SC}$ . This is because selfish mutants are individually more fit than cooperators and can be fixed by selection, whereas cooperative mutants can only be fixed by drift. However, the ratio  $P_{CS}/P_{SC}$  can be relatively small when populations are small due to resource limitation and/or when the costs of cooperation  $d_A$  and  $d_B$  are small. Both conditions can give rise to a selective regime that is nearly neutral (i.e. when cooperators are only slightly less fit than selfish types). Whatever this ratio, we can expect some portion of communities to be cooperative at any given time. If the ratio is  $P_{CS}/P_{SC} = 10$ , for example, then we can expect the metacommunity to reach a dynamic equilibrium with one cooperative community for every ten selfish communities at any one time. Starting with a metacommunity composed only of selfish types, we can therefore imagine cooperative communities episodically arising and disappearing over macroevolutionary time scales.

The above argument provides an explanation for the existence of cooperative communities with mutualistic flower-pollinator interactions based on mutation and genetic drift under a nearly neutral selective regime. However, to explain the *proliferation* and *fixation* of the cooperative variants in the metacommunity, as well as the subsequent coevolution of the mutualists leading to obligate mutualism, we must first assume a dispersal process. Consider that, in the absence of dispersal, the transition from a selfish to a mutualistic community by drift can take a very long time if single cooperative variants can only be introduced by chance mutation. The dispersal of cooperators into a selfish community, either by windborne migration in the case of flowers or trait-group selection in the case of pollinators, obviates the need to wait for a mutation to arise. Furthermore, when cooperative types enter a selfish community by dispersal, they do so with larger numbers and with greater frequency compared to single mutations. This combination of larger numbers and greater frequency shifts the dynamic equilibrium compared to a metacommunity

without dispersal in which cooperative communities are impermanent toward a state in which the cooperative variant is fixed in all communities.

Of course, the opposite is also true – a selfish type is more likely to be fixed in a cooperative population when many are introduced by dispersal compared to a single mutant. Here the assumption that the constituent populations of a cooperative community are larger than those of a selfish community is crucial. We can assume that the expected size of a dispersal “propagule”, generally defined to be those individuals drawn from one community that are to be transferred into another community, is proportional ( $p$ ) to the size of the community from which it was drawn. Hence, a windborne migration propagule composed of the seeds of cooperative flowers will be larger than a windborne migration propagule composed of the seeds of selfish flowers. Moreover, the cooperative migrants, when transferred into a selfish community, will face a relatively smaller population of selfish flowers. The selfish migrants, by contrast, will face a relatively larger population of cooperative flowers when transferred into a cooperative community. This is similarly the case for pollinators, which are assumed to disperse by trait-group selection where the dispersal propagule is the entire population ( $p = 1$ ).

The weight of numbers advantage enjoyed by cooperative communities therefore amplifies the probability that cooperative migrants will reach fixation when introduced into a selfish community and diminishes the probability that selfish migrants will reach fixation when introduced into a cooperative community. The larger a cooperative community is compared to a selfish community, the stronger these effects will be. This in turn will make it more likely that the mutualistic flower-pollinator relationship will be fixed in the metacommunity and remain so while the ecological features that support the MLS1 process remain in place (c.f. Jones et al. 2023)<sup>9</sup>. In this way, MLS1 can explain the proliferation and fixation of cooperative variants in a metacommunity (i.e., the *origin* of obligate mutualism). The cooperative metacommunity can subsequently resist selfish mutants only so long as the ecological conditions that gave rise to the MLS1 process (e.g., the metacommunity structure, dispersal processes, resource limitation) persist. Yet these conditions, if they persist long enough for the mutualistic relationship to be subsequently strengthened by coevolutionary processes (i.e., the *maintenance* of mutualism), can potentially lead to an obligate mutualistic relationship such as exists between the long-proboscis fly and the long-tubed irise<sup>10</sup>.

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<sup>9</sup> The simple model presented in this paper uses many of the same assumptions as Jones et al. 2023, which explores them through computer simulations.

<sup>10</sup> The process we describe here, whereby traits fixed by drift (e.g., the lengthening of the tube of the irise, the lengthening of the proboscis of the fly) are subsequently preserved by purifying selection (due to the transition to obligate mutualism), is reminiscent of accounts of constructive neutral evolution (CNE) that purport to explain some of the complexity of living systems at the molecular level without appealing to selection. Our verbal model is therefore consistent with the proposition that, like natural selection, CNE may be a general process that occurs at multiple levels

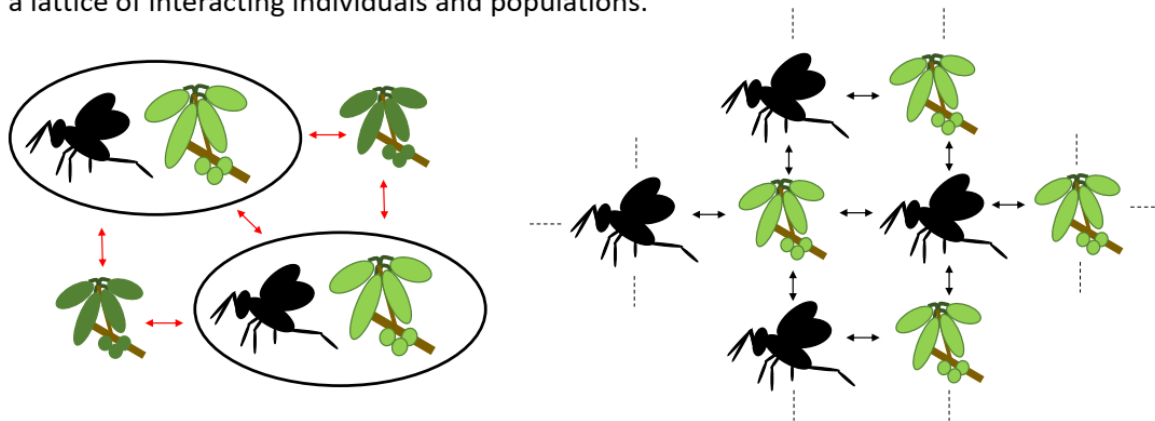
### 4.3 The Multispecies Population

There remains, however, the question of what mutualistic populations this model applies to. Not all individuals that interact with a set of mutualistic populations are members of a multispecies population. For example, in Wilson & Sober's (1989) phoretic association of beetles and their fungi, the systematic environmental danger posed by tree defences is overcome through their association conferring a fitness benefit to both the individual beetle and the fungi it spreads. This neat three population relationship involves two lower-level particles, the multispecies group of the beetle-fungi are bonded by their positive fitness relationship, and they have an antagonistic relationship to the tree (See Figure 2. on the left for this grouping in a Fig-Wasp Mutualism). This, however, is an idealization. As Wilson and Sober note, there are many other species involved in the phoretic association, with mites, nematodes, and microbiota interacting, in addition to many other outside species. The partially closed causal structure appears within an otherwise open lattice of causal relations, with some species in different strengths of association. This is a ubiquitous feature in ecological settings, where numerous species are constantly interacting in varied network topographies leading to the varied cohesion of ecological communities as a unit (Dunne et al. 2002, Lean 2018). Resources for describing the necessary population structure for the multispecies population can be found in the discussion of MLS1 in open lattices of interacting individuals (Godfrey-Smith 2008; Birch 2020). Note that individuals can evolve by MLS1 despite not forming distinctly bound groups (unlike MLS2), making MLS1 highly applicable to ecological settings.

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of biological organization, or at least that evolution can occur via a mix of CNE and adaptive selection (Brunet & Doolittle 2018).

**Figure 2. Groups versus Lattice:** In ecology population structure is not often ‘grouped’ but rather a lattice of interacting individuals and populations.



On the left we have a depiction of multispecies selection. The wasp and light green fig individuals form evolving multispecies populations, depicted by the circles, where their positive fitness interactions are strongly intertwined with their partner. This mutualistic group may compete with other conspecific groups or other individuals, represented by the dark green fig. On the right we have an open lattice structure. The positive interactions are not in the form of self-contained groups but rather dispersed among similar individuals who can participate in this relationship. There are no necessary boundaries to dictate the presence of ‘groups’.

A criterion is required for determining whether interacting species are part of the evolving multispecies community or interacting with it. We believe that such a criterion will have to be developed from previous single species population definitions. Identity conditions for complex evolving populations have been developed by Roberta Millstein (2006, 2009), Godfrey-Smith (2009), and John Matthewson (2015). There is variation in these authors' views, but the focus is on the causal mechanisms that maintain the *fitness affecting interactions* between individuals and the mechanisms that maintain *phenotypic stability* across that set of individuals<sup>11</sup>. Multispecies populations will be maintained by their fitness affecting interactions, and the ecological conditions that scaffold these interactions. Under our model, the population structure maintaining ecological features are dispersal and reassembly, which will allow for a stable altruistic interaction. The causal cohesion of the multiple interacting species will allow for consistent fitness interactions over evolutionary time and the strength of the conferred fitness benefit will determine the population boundaries.

Within multispecies populations, the relevant phenotype, that must be stable, is the multispecies phenotype of mutualistic interaction. The mechanisms that maintain altruistic interactions across both species define the boundaries of the relevant multispecies population. Phenotype is also stabilised through intra-species gene flow. Individuals in a multispecies population will not have gene flow across the different species but the other species may function as an environmental mechanism to maintain this gene flow,

<sup>11</sup> Stencil (2016) suggests that there can be multispecies Darwinian individuals but primarily focuses on fitness affecting interactions as his criteria.

creating between species genetic associations within the multispecies population. This is why in the following section we focus on mutualistic interactions where each species acts to foster the gene flow of the altruistic type. Precise multispecies population identity conditions remain future work for researching multispecies evolutionary dynamics.

This criterion may help distinguish between co-evolution and multilevel selection. Co-evolution and MLS1 are not exclusionary, the co-evolution of traits can be a result of multilevel selection. But co-evolution does not imply MLS2 or MLS1. Co-evolution is a process where populations evolve features in response to the other populations that comprise their environment. This can be a positive or negative feedback relationship. Leigh Van Valen's red queen hypothesis (1973) is a case of co-evolution where predator and prey or parasite and host must co-evolve at a similar rate to respond to each other's adaptations. Such interactions do not yield selection at the group level as they reduce the fecundity of the populations. Multispecies MLS1 is a sub-type of positive feedback co-evolutionary interactions where a relationship allows the multispecies population to increase in frequency and stabilises the interspecies fitness affecting interactions and their mutualistic phenotype. Infrequent fitness affecting interactions dispersed across a region, that lead to co-evolution (see Thompson 2019), do not meet the conditions for multilevel selection. Strong fitness affecting interactions between the populations and interspecific interactions that influence genetic composition in each species are in our view required to gain the group structure necessary.

In the following section, we apply our model to real-world populations by discussing two cases, which display different degrees of interdependencies between mutualistic partners. These two cases represent the extremes of mutualistic interdependency, a specialised obligate mutualistic relationship, and a generalist facultative relationship. In the first case, it is obligate mutualism where some within the population can cheat, exploiting the resource of their mutualistic partner without conferring a benefit. In the second, we consider generalist species that do not rely on their partner to survive but gain a fitness benefit from interacting with them. Such populations can easily "cheat" by using the resources of other mutualistic partners. In both cases, we explore whether population structure and dispersal acts in a way that supports the evolution of mutualism consistent with the MLS1 paradigm. We believe that the facultative relationship warrants the description of a co-evolutionary relationship while the obligate interaction could qualify as MLS1.



## 5. Mutualism and Multispecies MLS1

Having demonstrated the conditions under which multispecies MLS1 might occur, and how it might lead to a transition from a facultative mutualistic relationship to one that is obligatory for both species, the question that remains is how to identify mutualisms that did evolve in this way. A common difficulty in evolutionary biology is that a given outcome can often be explained in multiple ways. One way to choose between them is to appeal to parsimony and settle with the simplest explanation (e.g., Williams 1966). To propose a more complex explanation one must first demonstrate that the required conditions (e.g., a metacommunity structure, weight of number dispersal, and nutrient limitation leading to small populations with soft selection and strong drift) can arise in nature. Previously we have only claimed that multispecies MLS1 in a metacommunity provides a plausible explanation for the evolution of mutualisms provided the required conditions do exist, and appealed to rare cases of evolutionary transitions to multispecies MLS2 to provide circumstantial evidence for multispecies MLS1. In this section, we explore actual mutualistic relationships and suggest the conditions under which they could be subject to MLS1.

We consider two extremes of mutualistic interactions to illuminate the conditions under which our model may be applicable. To use concrete examples through this section, we contrast populations with high mutualistic dependency, such as those between many species of Figs and Wasps with weak mutualistic relationships with low mutual dependency, such as that between Bottlebrushes (*Callistemon rugulosus*) and New Holland Honey Eaters (*Phylidonyris novaehollandiae*).

Mutualist relationships can be specialised or generalist and obligate or facultative. Strong mutualist relationships are both specialised and obligate while weaker ones are both general and facultative. In an obligate mutualism, for each species or genotype to pass itself on to the next generation it requires the relevant interaction with its partner or partners (Chomicki et al. 2020). If it is a specialised mutualist, there is only one partner species. Obligate specialised mutualistic relationships, including the Fig-Wasp mutualism, where both species require each other for reproduction (Machado et al. 2001). Obligate generalist mutualisms include Bat-Cacti pollinators where the cacti require bat pollinators, but several bat species can pollinate the cacti (Nassar et al. 1997). The Callistemon-Honey Eater relationship is a generalist facultative mutualism as for either species or genotype to be passed on they do not require a mutualist partner, and they have many possible partners. We argue that on either side of the extremes of stable mutualistic interactions, the model will not apply but we provide the conditions under which this would change. Within generalist facultative relationships low interdependency of the populations can change given higher fitness interactions and patchy alternative resource options, leading to a shift towards

higher mutualist interdependency and MLS1. Within Obligate Specialised relationships, invasion by cheaters can be spontaneous in a population, MLS1 dynamics can then function as a factor in stabilising obligate mutualisms.

## 5.1 Obligate Specialised Mutualism

We started with the hypothesis that an important route through the transition from independent lineages to a new biological individual could be through MLS1. Therefore we start with mutualistic relationships that are closer to the transition to a selective regime with shared reproduction (i.e., MLS2). As argued in S. 3, a stable obligate mutualism will likely have transitioned past an MLS1 stage to reach a stable arrangement where co-evolution becomes the main evolutionary dynamic. This is because hyper-specialisation makes high-fitness impact cheating unlikely within stable obligate relationships and therefore the population is not subject to MLS1. However, the population dynamics established by obligate mutualisms maintain the conditions necessary for MLS1 if and when efficient cheaters arise in those lineages.

In many obligate specialised mutualist relationships, there is a strong entanglement of the reproduction of two species. For example, in the Fig and Wasp mutualistic pairing, the fig ‘fruit’ has internal flowers, the wasp burrows into the fig and lays its eggs and in the process fertilizes the flowers. The fig detects the presence of the eggs and surrounds them with a nutritious tissue that the larvae eat and upon maturity the fertilised female wasps burrow out taking the fig’s pollen with them, restarting the life cycle. Both species require each other for their reproduction, and the stability of their intra-species phenotype, and there is spatial continuity in the reproductive act in their pairing making it borderline vertical inheritance. This reproductive entanglement drives the community reassembly necessary in our model of MLS1. The nested hierarchical spatial structure of these mutualistic interactions could foster multilevel selection, as there is competition between wasps within flowers, wasp populations between flowers, and between the plants due to their association with wasp populations (Okasha 2006; Akçay 2015).

This entanglement can lead to dependencies that foster the diversification of the paired clades of species in the mutualistic relationship. We find whole genera with species in symmetrical mutualistic arrangements with the species of another genus. There are around 750 different mutualistic individual species pairings of Fig – Wasps and around 500 Cheese tree – Leafflower Moth relationships discovered

so far (Finch 2018). In these cases, it is difficult for species in these clades to evolve to become completely free-living as their reproductive function is dependent on the presence of their mutualistic partner species. But there are also clades with diverse mutualistic relationships. For example, Chomicki & Renner (2016) map a clade of mutualistic arrangements where epiphytic plants farm ant populations by supplying them with sugar rewards and housing in exchange for defence against herbivores. They find some epiphytes specialise their reward for one ant species while others provide general rewards supporting colonisation by multiple ant species and others lack mutualisms. This indicates that obligate mutualism is not a phylogenetic trap from which populations cannot escape. This possibility of defection allows for MLS1 dynamics to take hold in the population, requiring cyclic metacommunity dynamics to maintain mutualisms.

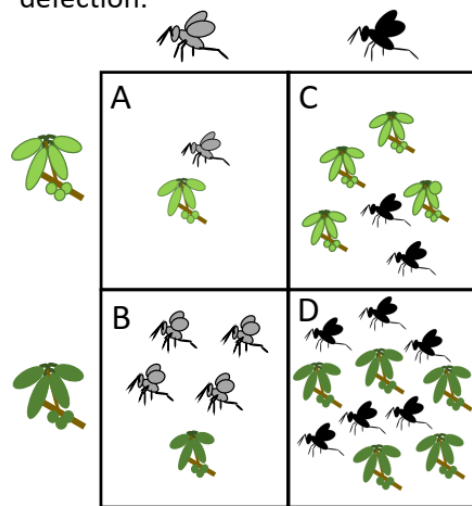
There are well-established cases where an obligate mutualistic partner defects, using their partner as a resource without conferring any benefit (Sachs & Simms 2006). Prominently researched examples include figs-wasps, *Yucca* cactus-moth interactions where individual flowers can be desiccated when they are not being pollinated by the moths, or legume-rhizobium mutualisms where individual root nodules can be starved of nutrients if the rhizobium does not supply nitrogen (Akçay & Simms 2011; Addicott & Tyre 1995). Within figs and wasps cheating arises either through the evolution of specific parasitic wasp taxa that lay their eggs but do not pollinate the fig or individual wasps may spontaneously display cheating within otherwise mutualistic populations (West et al. 1996; Jandér & Herre 2010). Given this, there is a constant chance of cheating within the obligate mutualism, which may sometimes confer enough of an advantage to reach fixation in a population. In response to cheating some fig species can detect whether its fruit has not been pollinated despite the presence of wasps, and in response will desiccate the fruit causing the death of the larvae inhabiting that fruit. Such mechanisms for cheater detection have costs and we will explore whether the mutualism can be maintained even when such mechanisms have not evolved.

This fitness interaction is represented in the model in Table 1. When a single community consists only of cooperative pollinators and selfish flowers, for example, the pollinator suffers a fitness deficit compared to a selfish pollinator (i.e., because there is a cost  $c_X$  to cooperation but no benefit in the absence of cooperative flowers). The cooperative pollinator population is therefore vulnerable to invasion by a selfish pollinator variant, which spontaneously arise in natural populations (Sachs & Simms 2006). The metacommunity structure with episodic exchange of individuals is maintained by the reproductive regime allowing for rapid dispersal and reassembly of the mutualistic interaction allowing for MLS1 to maintain mutualisms within our model. For weight-of-number dispersal in a metacommunity provides a

means to overcome cheaters despite their local advantage as we will elaborate in Figure 3. This curtails the need for one species, the fig for example, to hyperspecialize its adaptations to combat cheating wasps.

In these cases of coordinated reproduction with cheating variants, the dynamics are close to MLS2 given that there is a shared reproductive process. The dynamic of dispersal is however closer to MLS1 (in each metapopulation), and the coordinating unit is held together by the joint fitness relationships between the interacting species. Each species in the multispecies unit has a different temporal scale in its life cycle which affects how it disperses through the metacommunity. The difference in life cycle and dispersal mechanisms means that each species' fitness needs to be assessed separately despite forming a multispecies population. The multispecies population with little cheating will gain from weight-of-numbers dispersal, with each species sending out independent propagules to new patches with the hope of their mutualist partner catching up (See Figure 3.).

**Figure 3. Obligate Mutualism:** An obligatory mutualism with differences in fecundity due to defection.



Each species has two genetic variants (light-green tree = defection, dark-green tree = co-operation; gray wasp = defection, black wasp = cooperation). Wasp-fig mutualism is obligate, so the two species must exist together to reproduce. An individual wasp is more likely to reproduce when there are more fig trees, and an individual fig tree is more likely to reproduce when there are more wasps. Hence, the average fecundity of a wasp is a function not only of its own genes but also of contextual effects, the size of both the wasp population (since wasps compete for figs) and the figs population (more figs equals greater likelihood of reproduction). In the scenario depicted, the gray wasp provides very little pollination. This means it does poorly when paired with the light-green fig which does not supply nutrients to the pollinators in abundance and invests resources in strong policing mechanisms (A) but does better when paired with the more fecund dark-green fig but the dark-green fig has reduced fecundity due to a lack of pollination (B). The black wasp is more fecund but not as well with the light-green fig, the fig does well due to the pollination (C). The largest community is the one in which the black wasp is paired with the dark-green tree who both do not invest resources in defection/policing (D). Suppose there is a network of ecological patches that can each support a wasp-tree community, where all patches have the same level of resources. Weight-of-numbers dispersal (when a larger population disperses more effectively than a smaller population) can result in the proliferation of the black wasp plus dark-green tree combination across patches.

Within such scenarios, MLS1 rather than solely co-evolution may be a reasonable explanation for the stability of the mutualistic interactions. High rates of cheating and the consequent need to invest in policing by internal means in the absence of multilevel selection will reduce the allocation of resources toward reproduction. Cheating degrades the local fitness landscape for all within that population structure due to the competitive interactions. A metapopulation structure provides a means by which variation in the level of cooperation across communities can arise by selection and/or drift. Episodic weight-of-numbers dispersal provides a means by which selection at the community level can act on this variation and lead to the fixation of the cooperative variants of each species. Mechanisms for the reassembly of the

community, such as the ability to detect their mutualistic partner species or reproductive entanglement, ensure that the process of dispersal and reassembly can be maintained for circumstances where cheaters arise.

Through understanding this dynamic as MLS1, we can see the similarities between the intraspecies case and the interspecies cases of altruism. Instead of a hard gradient between co-evolution and transitions in the levels of organisation in which two lineages become a single reproducing lineage (MLS2), we can identify how group-level features can stabilise co-ordinated fitness interactions and allow for some species to create and maintain multispecies phenotypes. This is where each species, of the multispecies population, has individual adaptations that co-ordinate so they produce joint complex adaptive responses to environmental stressors. This could allow for the co-ordinated phenotypes in mutualistic multispecies populations, like for example the acacia-ant population phenotype of long hollow spines that house the ants (Sterelny 2011). The ants protect the acacia against herbivory with their painful bites and clear seeds of other species around the tree. In reply, the acacia supplies the ants with accommodation and food in the form of nectar secreted for the ants. Sterelny argues the adaptation, the ant-filled spines, is an extended phenotype of the ant and acacia genotype, a multispecies adaptive phenotype. MLS1 does not require ‘group-level’ adaptations, but the formation of adaptations, which appear to be the product of several distinct populations, is evidence that such selective regimes exist. The ability to provide an explanation of such complex adaptations at the multispecies level could provide a distinct advantage to considering the evolution of multispecies units, which are not reproduced together.

## 5.2. Facultative Generalist Mutualism

There is a strong case for mutualistic species with specialised reciprocal adaptations to be analysed as multispecies populations, but what about mutualisms where the fitness is not so strongly interdependent? To work through an example, consider the generalist facultative mutualism between Bottlebrushes (*Callistemon rugulosus*) and New Holland Honey Eaters (*Phylidonyris novaehollandiae*)<sup>12</sup>. For either species or genotype to be passed on they do not require a mutualist partner, and they have many possible partners. Bottlebrushes are largely self-incompatible and mostly require pollinators to successfully reproduce, which include birds, introduced European Honeybees, native Bees, Moths,

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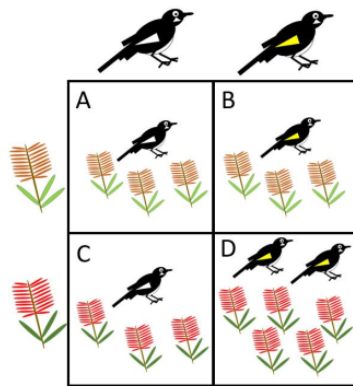
<sup>12</sup> Through the following text we will refer to New Holland Honey Eaters (*Phylidonyris novaehollandiae*) as Honey Eaters and (*Callistemon rugulosus*) as Bottlebrushes for ease of reading.

Possums, and Butterflies. Honey Eaters are equally in no way faithful to the nectaries of Callistemons and will consume nectar from Grevilleas, Banksias, and Gums, and consume small insects and spiders.

Unlike the case of Figs and Wasps, there is no strong reproductive interdependency or cost of defection that would bind the group structure together between generations. These populations could drift apart for many generations and there is no shared fate of these populations despite any fitness gain they might get from interaction because each mutualist population can exploit different resources. It may be more fitness-enhancing to spread independently, even if they exploit less optimal relationships. The utility of staying independent would normally swamp any possibility of MLS1 while maintaining fitness-affecting interactions that lead to co-evolution. Given this, there would also be no reason to invest in specific co-adaptations that would allow for the multispecies population to reassemble.

If the ecological conditions change, however, a generalist facultative mutualism could shift towards specialised obligate mutualistic interactions through MLS1. To create a hypothetical example; a sub-population of Callistemons and Honey Eaters could find that their interaction together has a higher fitness compared to the average individual that does not engage in this relationship. So, for this to be the case the Callistemon must have a much higher fitness by being visited by the Honey Eater compared to Bogong Moths and Honey Eaters a fitness gain from exploiting Callistemon nectar over that of Grevilleas. Such reciprocal gains in fitness would distinguish the subpopulation in that mutualistic relationship from all alternative subpopulations. This could be due to the appearance of a random mutation in the population or a change in ecological conditions, such as the patchy availability of other resources. This would create a large advantage for individuals that exploit this relationship over the other alternative pollination/ foraging relationships and allow for weight-of-numbers dispersal to increase the representation of the mutualistically paired species in the metacommunity (Figure 4).

**Figure 4. Facultative Mutualism:** Sub-populations in a species, with a high- fitness mutualism between one set of subpopulations.



Suppose each of the two callistemon subpopulations (red and brown) have the same average fecundity, and likewise for each of the honey eater subpopulations (yellow and white). Further suppose the red callistemon and yellow honey eater have evolved a facultative mutualistic relationship such that each increases the fecundity of the other.

Now imagine a network of ecological patches that can each support a callistemon-honey eater community, where all patches have the same level of resources, and all are occupied by a callistemon-honey eater community. There is no difference in the average fecundity of the two species in communities without mutualism (A, B and C). The average fecundity of the two species is amplified only in those communities in which the red callistemon and yellow honey eater subpopulations are paired together. Weight-of-numbers dispersal can support the proliferation of the red callistemon and yellow honey combination across patches.

Competition with other variants can arise in two ways, the first is with individuals that exploit alternative resources (heterospecific individuals) rather than the focal mutualist relationship, and the second is with cheaters (which are our focus). Pollen robbers and thieves are common in pollination mutualisms. Robbers pierce the nectary taking the nectar without exposing themselves to pollen and thieves enter the flower but avoid providing any pollination service (Irwin et al. 2001). In cases of direct competition with efficient cheaters, MLS1 can arise as in the scenario described in our model<sup>13</sup>. Direct competition between the mutualist and cheater may require significant changes in the ecological conditions to limit the alternative foraging options for the mutualist. If these are limited, then the direct competition could require MLS1 driven metacommunity dynamics to maintain the mutualism and can drive the further co-evolution of these facultative mutualists to become more specialised. This could ultimately lead to obligate mutualism.

In the case of competition with heterospecific morphs, there is not the same level of competition between the mutualistic paired unit and the individuals in alternative mutualistic interactions. This is because there is not the same degradation of the local fitness environment that occurs when the two variants compete. In facultative mutualisms, the variants exploit different resources. While there will still be competition over mates and other resources there is not the same level of competition over these resources to drive the need for metacommunity dynamics to maintain the focal mutualistic population. Both forms can co-exist in such situations. If competition rapidly increases due to a third limiting

<sup>13</sup> We say efficient cheaters as cheating may be a more inefficient foraging strategy than faithful foraging in some facultative mutualism (see Litchenberg et al 2018).

resource, such as nesting space, we believe there could be enough direct competition to allow for multilevel selection. This, however, would need further modelling. Our focus remains on the competition between cheater and mutualist morphs driving multispecies MLS1.

In normal circumstances, facultative mutualistic relationships can stay highly stable, due to the multiple realisation of functional ecological roles by multiple actors and the availability of alternative resources. But circumstances can change. Metacommunity pooling and dispersal, and patchy alternative resource availability, could lead to MLS1 and increasing adaptive specialisation of the two species ultimately trending them towards an obligate mutualistic interaction instead of the generalist interaction they currently exhibit. MLS1 may appear in times of environmental upheaval and be a significant evolutionary process in the ecological setting.

## 6. Conclusion

There is a wide acceptance that multiple species can fuse lineages and be subject to MLS2, but there has been little discussion of whether MLS1 can occur on multiple species. We have argued that there is good reason to believe that MLS1 occurs in multispecies arrangements. This would be a radical expansion of the presence of MLS1, from single species populations to multispecies populations. To do this we have proposed a model of multispecies multilevel selection. We indicate that in situations of high competition with cheater variants, a population of mutualists can be maintained through metacommunity dynamics of dispersal and reassembly. These conditions can allow for the evolution and optimisation of a mutualistic phenotype in the multispecies unit. Finally, we discussed actual mutualisms and proposed the environmental conditions that could ultimately drive co-evolutionary relationships into being subject to MLS1 and functioning evolutionarily as a multispecies population.

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