Are ant supercolonies crucibles of a new major transition in evolution?

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Abstract

The biological hierarchy of genes, cells, organisms and societies is a fundamental reality in the living world. This hierarchy of entities did not arise *ex nihilo* at the origin of life, but rather has been serially generated by a succession of critical events known as 'evolutionary transitions in individuality' (ETIs). Given the sequential nature of ETIs, it is natural to look for candidates to form the next hierarchical tier. We analyse claims that these candidates are found among 'supercolonies', ant populations in which discrete nests cooperate as part of a wider collective, in ways redolent of cells in a multicellular organism. Examining earlier empirical work and new data within the recently proposed 'Darwinian space' framework, we offer a novel analysis of the evolutionary status of supercolonies and show how certain key conditions might be satisfied in any future process transforming these collaborative networks into true Darwinian individuals.

Introduction

Several times since the origin of life, novel forms of individuality have emerged (Buss, 1987; Maynard Smith & Szathmary, 1995; Michod & Roze, 1997). Each of these events fashioned new levels on the biological hierarchy, from genes and cells to multicellular organisms and eusocial societies, renegotiating the parameters within which natural selection operates (Godfrey-Smith, 2009).

The sequence of such 'evolutionary transitions in individuality' (ETIs) is the unifying narrative in the history of life. It is therefore of considerable interest to examine candidates for the next transition (Bourke, 2011). In certain ant populations, nests are not mutually aggressive and indeed actively cooperate and communicate. These so-called 'supercolonies' have been proposed as candidates for future individuation (McShea, 2001; McShea & Changizi, 2003; Bourke, 2011; Pedersen, 2012), but a thorough evaluation of this possibility has until now

been lacking. We synthesize research on ant supercolonies from an explicitly ETI perspective and introduce new data to resolve questions raised by this framework.

There has been much debate as to the definition of 'supercolony' (Gordon & Heller, 2012; Lester & Gruber, 2012; Moffett, 2012a,b; Pedersen, 2012; Suarez & Suhr, 2012); here, we use the term in its conventional sense for a large network of functionally integrated polygynous (multiple queen) nests (Helanterä, 2009). Workers, brood, queens and resources are trafficked between these nests. Consequently, individuals regularly cooperate with nonrelatives. This not only appears paradoxical for inclusive fitness theory (Jackson, 2007; Helanterä *et al.*, 2009), but also suggests supercolonies may be vulnerable to trait degradation – as the extensive mixing of lineages that leads to workers helping unrelated queens should hide worker phenotypes from selection – and therefore be evolutionarily short-lived (Queller & Strassmann, 1998; Linksvayer & Wade, 2009; Helanterä *et al.*, 2009; note that this may not apply in supercolonial species with worker reproduction, for example in some *Formica*, Helanterä & Sundström, 2007). However, competition among adjacent supercolonies (Pedersen *et al.*, 2006) or genetic viscosity within supercolonies in some genera (e.g. *Formica*) may restore the utility of altruism (Chapuisat *et al.*, 1997; Helanterä, 2009; Holzer

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et al., 2009), potentially maintaining supercolonies as stable social structures that could be candidates for individuality.

Godfrey-Smith (2009) recently introduced a powerful new framework for conceptualizing Darwinian processes, with immediate application to ETIs. This paradigm has received much attention in the philosophy of biology (e.g. Dennett, 2011; Pradeu, 2011; Sterelny, 2011), but has so far not received widespread application among biologists. Different populations are said to occupy different locations in a hyperdimensional space, where the dimensions are parameters relevant to the degree to which populations can evolve through natural selection (Table 1). Populations can, over time, traverse this 'Darwinian space' (Fig. 1). In so doing, they may assume the characteristics of 'paradigmatic' Darwinian populations, or, indeed, lose them. A 'Darwinian population' is simply a population capable of evolving by natural selection, and a 'Darwinian individual' is a member of such a population (Godfrey-Smith, 2009).

This framework's major contribution to the study of ETIs is the concept of 'de-Darwinizers', defined as those features that reduce the potential for evolution by natural selection to occur within a population (Godfrey-Smith, 2009). During an ETI, selection stalls within a Darwinian population, and the population becomes itself an individual in a higher-level Darwinian population. For instance, as multicellularity evolved, selection between cells was greatly reduced, and selection between multicellular groups arose (Grosberg & Strathmann, 2007). Key de-Darwinizers emerged and drove this individuation, including germ-soma separation (the Weismann Barrier) and bottlenecked life cycles (Grosberg & Strathmann, 2007).

A supercolony can be decomposed into separate levels. At the highest level is the supercolony itself;
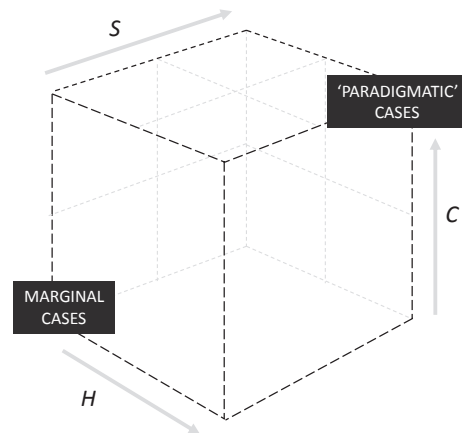
Fig. 1 Godfrey-Smith's (2009) 'Darwinian space' framework places populations in a hyperdimensional space, where the dimensions (including, but not limited to, *S*, *C* and *H*) define the extent to which evolution by natural selection can operate within the population. Adapted with permission from Godfrey-Smith (2009).

beneath this are the individual polygynous nests, and within each nest are multiple matrilines. To examine the context within which any potential ETI might operate, we evaluate these tiers according to the key parameters of Darwinian populations and relevant 'de-Darwinizers'. Having clarified this context, we show the possible trajectories for individuation of the supercolony. We suggest that these nest collectives are analogous to sponges in their social organization.

Framing the question

Monogynous (single queen) eusocial societies can be treated as Darwinian individuals (Haber, 2013).

Table 1 Relevant parameters in the 'Darwinian space' framework. There may be significant differences between native and invasive supercolonies in these parameters.

Parameter	Definition	Example data on supercolonial species
B	Extent to which life cycle involves a bottleneck	Hee *et al.* (2000) experimentally test the role of propagule size in invasive *Linepithema humile*. Seppä *et al.* (2012) use mtDNA to reveal the queen colonization history of different native *Formica* supercolonies
G	Extent of germ-soma separation	Currently no data
I	Extent of integration between constituent parts	Some data on functional integration (e.g. Holway & Case, 2000). Much evidence of reproductive behaviour occurring in only a few nests in polydomous populations (e.g. Scherba, 1961; Ito & Imamura, 1974; Kim & Murakimi, 1980; Pamilo & Rosengren, 1983)
V	Abundance of variation	Various authors have shown between-supercolony variation (e.g. Buczkowski *et al.*, 2004 show aggression differences), but the extent to which variation is genetic remains unknown
α	Extent of competitive interactions in relation to reproduction	Currently no data
H	Fidelity of heredity	Currently no data
C	Smoothness of the fitness landscape	Currently no data
S	Extent to which variation in fitness arises from traits intrinsic to entities themselves	Currently no data

Functionally integrated, with clear germ-soma separation between fertile queens and sterile workers (Boomsma, 2009), they proceed through a lifecycle bottleneck when founded by a single young queen (haplometrosis). The descendants of such monogynous colonies are found today in supercolonies as matrilines, with multiple matrilines cohabiting a single nest. If an ETI is to be had, is the relevant lower-level population for 'de-Darwinization' the matrilines or the archipelago of nests? In other words, what is the candidate individual: a polygynous nest or a supercolony?

We argue that the answer is a matter of perspective. The evolution of polygyny – whether by aggregation of nonrelated matrilines or adoption of daughter queens – generates a cohesive social group uniting multiple Darwinian individuals. Compelling examples are found in colonies of the ponerine *Pachycondyla inversa*, which are comprised of unrelated matrilines carrying out different functions (Helanterä *et al.*, 2013), in the simple unification of family groups by the fusion of distinct colonies in the termite *Zootermopsis nevadensis* (Howard *et al.*, 2013), and the recruitment of kin as queens in many taxa (Bourke & Franks, 1995). However, as we wish to examine the proposal that supercolonies represent a new ETI (McShea, 2001; McShea & Changizi, 2003; Bourke, 2011; Pedersen, 2012), here we address the candidacy of supercolonies as opposed to polygyny *per se*. In this study, then, we are concerned with the collaboration of polygynous social groups (nests) to form a wider collective.

When examining potentially ambiguous cases of individuality, an important distinction should be made between the Darwinian space framework and a recent approach based on fitness maximization (Gardner & Grafen, 2009). In the latter, the relevant feature for a collective to be considered an individual is not membership of a Darwinian population, but rather the state of being an agent-like group acting to maximize group fitness (Gardner & Grafen, 2009; Gardner, 2013). In Gardner and Grafen's (2009) approach, within-group clonality or the complete repression of within-group competition are the key determinants of individuality: without one of these conditions, within-group selection prevents group adaptation because the group does not fulfil the criterion of a maximizing agent in a formal inclusive fitness framework (for a less restrictive vision also based on group-level optimality, see Akçay & Van Cleve, 2012). Darwinian space answers a different question, and the word 'individuality' is invoked in a different sense by each framework. In this study, we will be concerned with the degree to which a population of entities can evolve by natural selection: 'Darwinian individuality' will be understood as membership of such a population. Crucially, Darwinian space allows us to see individuality as a continuous variable: there are greater and lesser degrees of individuality, as opposed to a discrete threshold.

Key parameters

In 'part one', we discuss the continuous parameters characterizing Darwinian individuals (Table 1). Specifically, these are the extent to which an entity's life cycle undergoes a bottleneck (B), the extent to which a germ line is sequestered from the soma (G) and the extent to which the candidate individual is an integrated, cohesive entity (I). In 'part two', we turn to the continuous parameters characterizing the Darwinian populations to which these individuals belong. Specifically, these are the abundance of variation in the population (V), the intensity of competition in the population (α), the fidelity of heredity among members of the population (H), the extent to which fitness differences in the population depend upon intrinsic features of individuals (S) and the smoothness of the fitness landscape (C). Many of these features resonate directly with formal quantitative measures of selection. We can, for instance, unify phenotypic variation (V) with fitness variation to derive the 'opportunity for selection' and derive from this the 'response to selection' by incorporating heritability (one practical measure of H) (Griesemer, 2000; Bijma *et al.*, 2007).

In accordance with the Darwinian space framework, we term the population of nests the 'lower level' and the population of supercolonies the 'higher level'. We rely both on published data from supercolonies of several ant species (largely focussing on native, as opposed to invasive, populations) and our own original data from six species of *Formica*, where we explicitly compare critical features among supercolonies and phylogenetically close nonsupercolonial populations (details of which can be found in the Supplementary Information).

Part One

B: Extent of bottlenecks

In common with many authors, Godfrey-Smith (2009) highlights the extent to which a life cycle proceeds via a bottleneck (B) as important to individuation. This is for two reasons.

First, a multicellular life cycle with high B creates an organism with clonal cells, short-circuiting natural selection (Dawkins, 1982; Grosberg & Strathmann, 2007). In terms of Darwinian space, high B generates low V ('abundance of variation'), a common feature of de-Darwinized populations. Paradigmatic Darwinian populations have ample variation for selection (Godfrey-Smith, 2009). Although V is arguably concerned with a number of diverse biological phenomena (Plutynski, 2010), minimal V in an obvious sense is crucial to a famous and arresting definition of individuality as genetic uniformity, vividly illustrated by Janzen's (1977) claims that a clonal group of aphids is in reality a

single individual and that (as strikingly paraphrased by Dawkins, 1982: 254) 'there may be as few as four individual dandelions in the whole of North America'.

Second, high *B* allows phenotypic novelty arising in the germ line to be distributed throughout the entire somatic tissue of the post-bottleneck individual (Dawkins, 1982; Godfrey-Smith, 2009), with the unique evolutionary feature that each individual allows a 'return to the drawing board' (Dawkins, 1982: 259).

B at the lower level (nest)
A typical single-foundress nonsupercolonial ('multicolonial') nest proceeds through a bottleneck (high *B*), with dispersal and lone-founding predominating. In a supercolony, as ants and resources are drawn from the wider network to form a fluid nest population (Moffett, 2012a; Hoffman, 2014), nests do not enjoy high *B* and thus their individuality is partially dissolved.

B at the higher level (supercolony)
The supercolony as a whole resembles a sponge, in that it arguably has more than one mode of reproduction: it may fragment or dispatch small propagules. Bottlenecks probably *do* meaningfully occur in supercolonies, as a result of jump dispersal by lone foundress queens or nest fragments containing multiple queens (Hee *et al.*, 2000; van der Hammen *et al.*, 2002; Abbott, 2006; Moffett, 2012a; Seppä *et al.*, 2012), although their presumed capacity to promote the evolution of invasive supercoloniality now seems unlikely (Giraud *et al.*, 2002).

Supercolonies are often long-lived, and – although new queens almost always come from within the supercolony – genetic diversity may be generated by the occasional arrival of foreign reproductives by long-range dispersal (van der Hammen *et al.*, 2002; Seppä *et al.*, 2012). Consequently, even with bottlenecks at the birth of every supercolony, high relatedness and genetic homogeneity will be eroded by these secondary arrivals. The maintenance of genetic homogeneity in a supercolony may thus be very different in native and invasive supercolonies, due to variation in the number of neighbouring supercolonies. In certain species, supercolonies may enjoy sufficiently high levels of isolation that their genetic identity is successfully preserved (e.g. *Anoplolepis gracilipes*; Drescher *et al.*, 2010).

In reality, the absence of violent struggles with non-relatives may – in many species – be an adaptive strategy to avoid the heavy costs of conflict, especially when information about kinship is poor (Helanterä, 2009), rather than a product of high *B* alone. The upshot is that although *B* is not high enough to generate high relatedness, idiosyncrasies of supercolonial life, particularly the low levels of genetic structuring and kinship information, may be sufficiently powerful to prevent destabilizing conflict among units of the lower-level population. Whereas cohesive individuals have, in

previous ETIs, likely been *generated* by kin selection (Fisher *et al.*, 2013), a supercolonial ETI may only be possible because kin selection may have been partially thwarted.

In many species, jump dispersal appears to be key to the formation of new supercolonies (e.g. Suarez *et al.*, 2001; Vogel *et al.*, 2009). A jump-dispersing queen can be seen as a unitary propagule in an ontogeny culminating in mitotic-like somatic growth (budding). Yet the bottleneck through which a supercolony passes is very different from that envisaged by Godfrey-Smith: it does not ensure homogeneity at the lower level over the lifetime of the supercolony, and thus, supercolonies default a common criterion of individuality. However, we also argue that this barrier to individuality – genetic mosaicism across the supercolony – may be negotiable, especially given the high turnover of native supercolonies of *Linepithema humile* (Vogel *et al.*, 2009). If the emergence of lower-level mosaicism were negligible as a result of short supercolony lifespan, *B* would rise. Moreover, scales of mosaicism are not necessarily the same as scales of competition: genetic mosaicism within a supercolony may not be problematic to supercolonial individuation if supercolonies are competing against genetically distinct supercolonies (see *α*, below).

Ereshefsky and Pedroso (2013) have argued that the second function of high *B* – distributing novelty – is superfluous to a definition of individuality, citing the prevalence of lateral gene transfer in biofilms as a process spreading innovations despite low *B*. There may be reasons to be sceptical of biofilm individuality, but lateral gene transfer is directly reminiscent of traffic between nests within a supercolony. Thus, even if a supercolony 'reproduces' by fission, fragmenting due to the attenuation of internest links, it can still distribute phenotypic innovations across the soma from distant nests; indeed, it could be said to be more effective at this than a paradigmatic multicellular individual, as the diffusion of ants (and consequently phenotypic novelty) away from their natal nest has no parallel in multicellularity. This process could be seen as a superorganismal version of 'pangenesis', Darwin's (1868) erroneous hypothesis for a mechanism of inheritance by which somatic cells are invited to dispatch hereditary information to the germ line.

G: Germ-soma separation

The sequestration of a germ line (high *G*) closes reproductive options for somatic entities, aligning their fitness interests with the germ line's and thus de-Darwinizing the lower-level population.

Without high *G* or high *I* (integration), high *B* is simply arbitrariness as to which member of a loosely formed group reproduces: as Godfrey-Smith (2009: 106) remarks, 'if only one pregnant buffalo makes it through the gap, that alone does not make herds into

Darwinian individuals'. Despite this, high G is more a feature of multicellular organismality than individuality *per se*: a unicellular organism clearly lacks high G, for instance. Yet elevated G does play a significant role by 'exporting' fitness to the higher level, such that it pays a member of the soma to invest in group fitness (Michod, 2005). Like high B, high G is a significant de-Darwinizer, rendering the vast majority of the lower-level population evolutionarily inconsequential in the longer term as selfish reproducers (Godfrey-Smith, 2011). Germ-soma separation is therefore a driver and symptom of individuality, but not a necessary criterion (Buss, 1987; but see Michod, 2011).

G at the lower level (nest)

Both nonsupercolonial and supercolonial nests are characterized by a strict reproductive division of labour (Fig. 2) and hence high G (Boomsma, 2009): worker reproduction notwithstanding, the germ line is entirely sequestered in gynes and drones.

G at the higher level (supercolony)

It is initially difficult to see what germ-soma separation in a supercolony might look like. A germ line must be totipotent to the extent of being capable of reproducing an entire supercolony, whereas the soma is differentiated. Germline nests must, therefore, be queenright. However, it is not clear that the soma should be queenless, although queenless nests may be sufficient to define a supercolonial soma if somatic growth is understood simply as the spatial spread of the supercolony. Conversely, queenright nests can still be somatic, if they function only in somatic growth and not in reproduction at the level of the supercolony. Moreover, nests that give rise to fragments capable of fissioning to produce novel supercolonies must be queenright.

In reality, it is likely that reproductive totipotency is maintained across the majority of the supercolony: almost any nest may found a new supercolony, with the significant exception of queenless foraging outstations. If queenlessness (and thus reproductive impotence) is a *sufficient* but not necessary condition for the soma, then the evolution of high G in supercolonies might occur by their evolving the extreme combination of polydomy and queen localization (in a single nest) that characterizes the nonsupercolonial species *Camponotus gigas* (e.g. Pfeiffer & Linsenmair, 1998). Today's supercolonies lack such sequestration (Fig. 2).

New supercolonies may be produced by fragmenting due to local environmental changes, in a manner analogous to fission in, for example, asexual planarian flatworms. If such fragmentation were more common in a certain type of nest, or if certain nests specialized in producing queens for jump dispersal, we might identify a germ line. At present, we presume that the apparent lack of terminal differentiation means that G is minimal in supercolonies.
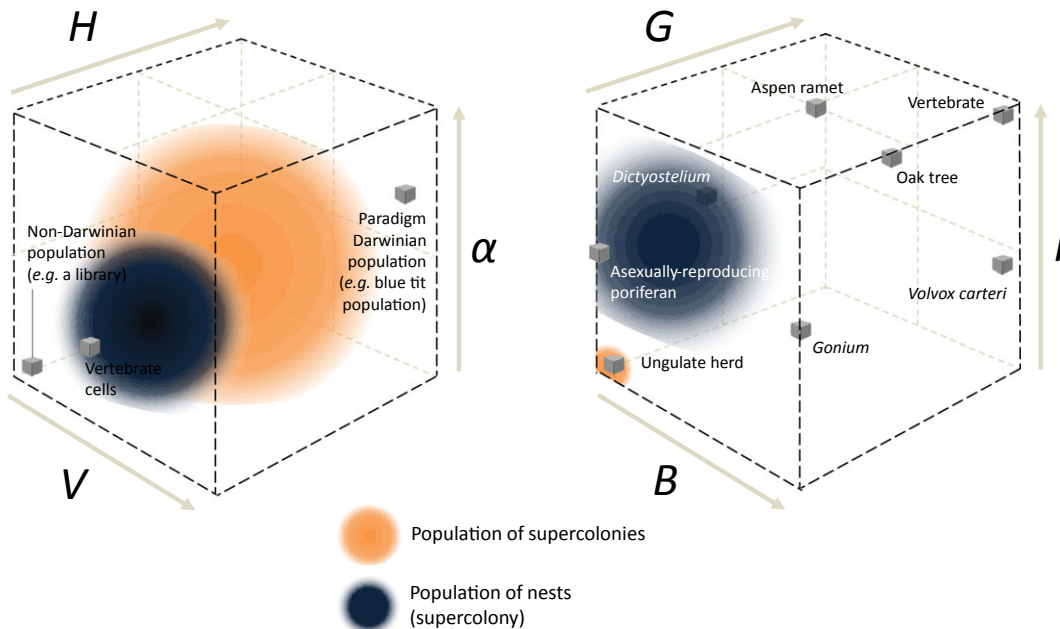
Fig. 2 Parametizing supercolonies according to Godfrey-Smith's (2009) 'Darwinian space' framework. V: abundance of variation; α: intensity of competition; H: fidelity of heredity; G: extent of germ-soma separation; B: extent of bottlenecks in the life cycle; I: extent of integration in the lower-level population. Our approximate positioning of the populations above is defended in the text. Right-hand diagram adapted with permission from Godfrey-Smith (2009).

I: Integration

The imaginary buffalo herd alluded to above may pass through a bottleneck (high *B*), but still fail to be an individual in its own right. Yet if we imagine two buffalo clones, physically attached, mutually codependent and functioning synergistically together, we might be happier to accept that this entity is a Darwinian individual. Godfrey-Smith's *I* parameter is consciously vague, but appeals to the intuition that strong functional association between entities strengthens the case for individuality, if it is coupled with membership of a higher-level Darwinian population. Anything with high *I* – such as a haemoglobin molecule (Godfrey-Smith, 2009) – is not composed of autonomous entities and thus is not helpfully treated as a population. High *I* is a feature of individuality in a general sense, a necessary but not sufficient feature of Darwinian individuals.

I is, then, a composite of a number of traits (Godfrey-Smith, 2009). Here, we separate these into (1) loss of autonomy and totipotency (I_{at}), (2) boundary development (I_{bo}) and (3) nonreproductive division of labour (I_{dv}).

I_{at}: Loss of autonomy and totipotency

Within the lower level (nest). Loss of reproductive and behavioural totipotency among sterile workers is a defining feature of eusocial societies (Crespi & Yanega, 1995) and does not differ between nonsupercolonial and supercolonial nests.

Within the higher level (supercolony). Clearly, if supercolonies endure a single-nest bottleneck, single nests are capable of surviving and reproducing. The question is whether an ontogenetic rise in nest number leads to mutual interdependence in large, mature supercolonies.

If the constituent parts of a genuine individual are atomized, they should be incapable of functioning. Thus, the simplest empirical test of individuality would be to divide the apparent individual and monitor the survival and functioning of its components.

This test is easily experimentally tractable by isolating nests from neighbours, and a similar experiment has, remarkably, been carried out on the supercolonial species *Formica polyctena* by translocating nests onto isolated, semi-barren islands (Czechowski & Vepsäläinen, 2009). A single nest has survived continuously on a small outcrop since 1987, although ambient conditions have apparently been too poor for the colony to produce its own sexuals; these are seemingly recruited from nests across the water (Czechowski & Vepsäläinen, 2009). This lack of autonomy is likely due to poor environmental conditions (and consequently poor foraging conditions) and it is possible that sexual production would occur if this colony were able to expand into novel territory and produce additional nests. Neverthe-

less, it is possible that single nests of this species are not reproductively competent.

Limited production of sexuals could be symptomatic of a general trend towards interdependence and integration between nests (Bourke, 2011), that is, high I_{at}. In a pairwise comparison between a supercolony of *Formica aquilonia* and a nonsupercolonial population of closely related *Formica pratensis* (see Goropashnaya *et al.*, 2012 for phylogeny), we found that reproductive activity in the supercolony is circumscribed to only 54% of active nests, despite being universal in its nonsupercolonial counterpart (Supplementary Online Material). This confirms that in polydomous populations, the production of sexual ants is often confined to a fraction of nests (Scherba, 1961; Ito & Imamura, 1974; Kim & Murakimi, 1980; Pamilo & Rosengren, 1983). Previously, sexual production has been shown to occur in only the larger nests (Kim & Murakimi, 1980; Cherix *et al.*, 1991); in our population, sexual-producing and non-sexual-producing nests were not significantly different in nest volume ($t_{67} = -0.15$, $P = 0.80$) and were similarly dispersed in the population (see Data S1 and Fig. S1 in Supplementary Online Material).

I_{bo}: Boundary development

At the lower level (nest). Nonsupercolonial nests can exhibit strong territoriality based on effective nestmate recognition strategies. In contrast, supercolonial nests are by definition more fluid, less definable structures, characterized by constant traffic of workers to and from neighbour nests.

At the higher level (supercolony). Gene flow between supercolonies can be minimal (Pedersen *et al.*, 2006; Vogel *et al.*, 2009), and members of foreign supercolonies can be recognized and aggressed (Thomas *et al.*, 2006, 2007). Thus, supercolonies are discrete units.

I_{dv}: Nonreproductive division of labour

Within the lower level (nest). One critical form of integration, division of labour, has loomed large in ETIs (Maynard Smith & Szathmary, 1995; Grosberg & Strathmann, 2007; Gavrilets, 2010). Both nonsupercolonial and supercolonial nests are characterized by a high level of internal division of labour among workers, illustrated, for instance, by the sophisticated resource processing of attine leaf-cutters (e.g. Hart & Ratnieks, 2001) and the brood care strategies of *L. humile* (Libbrecht & Keller, 2012), respectively. Task partitioning within the workforce (e.g. Hart *et al.*, 2002) constitutes a further genre of functional integration in nests. Moreover, workers can sophisticatedly integrate relevant information for task-based decision-making (Robinson *et al.*, 2012) and can show considerable and effective behavioural plasticity (Pinter-Wollman *et al.*, 2012).

The complexity of division of labour and task coordination in social insect nests is sufficiently remarkable that the relevant fields are referred to as 'social anatomy' and 'social physiology', respectively (Seeley, 2009; Johnson & Linksvayer, 2010), consciously evoking the functional complexity of a multicellular organism. Lastly, artificial selection experiments suggest that between-colony selection is capable of reconfiguring the colony 'sociogenome' regulating such higher-level physiological processes (Linksvayer *et al.*, 2009).

Within the higher level (supercolony). The internal structure of supercolonies remains little known (Debout *et al.*, 2007; Moffett, 2012a; Ellis & Robinson, 2014), and it has generally been assumed that supercolonies lack among-nests division of labour (Bourke, 2011; McShea & Simpson, 2011). However, the discovery of flexible allocation of workers to nests has led to speculation that such specialization does occur (Holway & Case, 2000). There are clear precedents: in *C. gigas* – a polydomous, albeit monogynous, species – peripheral forager nests act as metabolic 'sources' funnelling resources to the reproductive centre, an energetic 'sink' (Pfeiffer & Linsenmair, 1998).

The fixation of plastic responses across spatial gradients may have driven multicellular differentiation (Schlichting, 2003), as well as task allocation in colonial urochordates, cnidarians and bryozoans (Harvell, 1994). Similarly, nests throughout the supercolony are likely to experience different ambient conditions, which might propel any specialization. Systematic spatial differences may also arise in biotic interactions: interspecific competitors can be excluded from the interiors of supercolonies (Drescher *et al.*, 2011), and contact zones with rival supercolonies are likely to be flashpoints for aggression (Thomas *et al.*, 2006).

Functional division of labour can emerge in a homogeneous field of modules and generate 'stickiness' that raises the fitness gains of working together (Ispolatov *et al.*, 2011). Of relevance here is the enigmatic relocation of broods along the arterial highways of the supercolony. Trafficking future workers to the frontier may improve colonization capacity (Espadaler *et al.*, 2004) or allow the ensemble to track a spatially dynamic food resource (McIver, 1991). In an elegant experiment, Holway and Case (2000) demonstrated that *L. humile* workers translocate brood towards the sources of optimum brood-rearing resources. Accordingly, one explanation for our brood results above would be that sexual pupae are relocated to specific crèche nests, where they enjoy bespoke care, and are supplied with the appropriate nutrients. Such relocation would be facilitated by supercolonies' unique mode of 'dispersed central-place foraging' and might underpin division of labour between nests.

As well as analysing supercolonies for variation in the production of sexuals (see above), we also explored the supercolonial phenotype, comparing worker attributes between nests in three supercolonies. To provide a null expectation, we carried out the same tests on three nonsupercolonial populations phylogenetically close to these species (see Supporting Online Information for details). A heterogeneous lower-level population may reflect reduced integration; however, if it has functional implications at the higher level, it may be a feature of increased integration.

Firstly, using landmark-based geometric morphometrics on worker faces, we found – contrary to expectation – that worker size showed among-nests variation in all populations except two of the three supercolonies (see Table S1 in the Supporting Online Information). In all significant populations, effect sizes (η^2) were substantially higher than the heuristic cut-off for a 'large magnitude' effect (Cohen *et al.*, 2007).

The two supercolonies for which we did not find among-nests differentiation in worker size (*Formica fennica* and *Formica truncorum*) are distinct from the *F. aquilonia* supercolony in their extraordinarily high nest density. Mean *F. fennica* nearest-neighbour distance was 1.56 m (SE $\pm$ 0.31), in contrast to nearest-neighbour distances ranging over tens of metres in *F. aquilonia*. If increasing isolation limits among-nests worker traffic, we should expect the indigenous worker populations of more proximate nests to be more diluted by non-nestmates, although there was no significant correlation between the size differences and spatial distance between nests in *F. aquilonia* (Mantel's $r = -0.19$, $P = 0.37$).

We also analysed worker facial shape and found that it too differed significantly between nests in supercolonial *F. fennica*, *F. aquilonia* and *F. truncorum* (see Table S2), although effect size was negligible in *F. aquilonia*. Such between-nests variation is a surprisingly counter-intuitive result, especially within the *F. truncorum* supercolony, where nests are extremely interconnected.

A negative correlation between nest density and among-nests genetic differentiation has been reported in *L. humile* supercolonies (Ingram, 2002). Our results may, however, also reflect plasticity: widely spaced supercolonies stretch far enough to encompass environmental heterogeneity, and worker size in *F. aquilonia* is associated with environmental quality (Fedoseeva, 2011). We tentatively suggest that such phenotypic differentiation between supercolonial nests may have functional implications. However, in an assay of these supercolonies looking for nest specialization in aggression (see Data S1 for methods), we found no among-nests differences (see Table S3); similarly, analysis of foraging (see Data S1 for methods) found that supercolonies did not show elevated between-nests variance in foraging preference or intensity when compared with phylogenetically close nonsupercolonial populations (see Tables S4 and S5). Such apparent lack of division

of labour suggests a lower value for *I*. It is possible, however, that nest-level phenotypic differences may be relevant in explaining why sexual production appears limited to certain nests (see above).

Summary of I at the lower level (nest)
On the continuous spectrum sketched by Godfrey-Smith (2009), a nonsupercolonial nest lies somewhere between a car's engine and an orchestra in terms of *I*. It is not so tightly associated that its component parts are critically codependent and cannot be interchanged, but it does display synergistic division of labour and common 'purpose' (Anderson & McShea, 2001). The internal environment generated by 'social homoeostasis' is remarkably stable (Hölldobler, 1990), to the extent that nests have been termed 'homoeostatic fortresses' (Hughes *et al.*, 2008). A supercolonial nest is similar, but closer to the orchestra: its parts are in constant flux and regularly interchanged (Fig. 2).

Summary of I at the higher level (supercolony)
Minimal *I* is shown by populations such as gases in Brownian motion (Godfrey-Smith, 2009). Supercolonies are significantly more integrated (Fig. 2): using a dispersed form of central-place foraging, they share resources between nests (Holway & Case, 2000); they show features associated with efficient networks (Cook *et al.*, 2014); they show self–nonself discrimination with other supercolonies (Thomas *et al.*, 2006, 2007); there can be significant overlap in nest foraging zones (Erós *et al.*, 2009); nutritional exchange between nests has been treated as a homoeostatic mechanism (Rosengren & Pamilo, 1983); and they appear to relocate brood, workers and queens strategically (e.g. Buczkowski & Bennett, 2008). These consequences of nest budding are significant, as budding resembles incomplete cytokinesis, itself probably a crucial step in the evolution of volvocine multicellularity (e.g. Kirk, 2005; Herron & Michod, 2007).

We advocate dissecting supercolonies as though they were colonial metazoans. In various ways they resemble sponges, with traits likely to have characterized the ancestor of the Metazoa (e.g. Müller *et al.*, 2003): they are modular, between-nest traffic is analogous to cell–cell adhesion molecules, and intersupercolonial aggression is analogous to histoincompatibility.

Such integration, however, may be evolutionarily precarious. Supercolonies may show viscous substructuring within the population, such that relatedness is not zero throughout (Holzer *et al.*, 2009), and they may not be as devoid of subtle nepotism as previously assumed (Helanterä, 2009; Helanterä *et al.*, 2009). Peaceful integration between these clusters may be a convenience to avoid the high costs of conflict, and in particular the high cost of recognition errors. Ants in *Formica paralugubris* supercolonies have even been shown to engage in trophallactic food exchange with non-nestmates over twice as frequently as with nestmates, potentially suggesting that supercolonial stability depends on delicate 'appeasement' policies towards other nests (Chapuisat *et al.*, 2004).

Part two

The above parameters (*B*, *G* and *I*) describe qualities of individuals. We now turn to those parameters that describe the populations such individuals must inhabit if they are to be involved in evolution by natural selection (*V*, α, *H*, *C* and *S*). Data on these parameters are scarce, and our discussion is therefore by necessity brief.

V: Abundance of variation

Selection, of course, requires variation. In Darwinian space, the abundance of such variation is captured in the parameter *V*. For there to be *heritable* variation in *fitness*, there must also be high values for *H* and *S*; similarly, *V* coupled with phenotype–fitness covariance underpins the 'opportunity for selection' (variance in relative fitness; Crow, 1958).

V at the lower level (nest)
Our data (above) show ways in which the nests both within and outside supercolonies can vary in phenotypic traits. Nonsupercolonial nests are known to show variation (e.g. Gordon, 2013).

V at the higher level (supercolony)
There is suggestive evidence that supercolonies can show significant intraspecific variation (Fig. 2), varying widely in their territory size and nest number (van der Hammen *et al.*, 2002; Abbott, 2006), recognition cues (Drescher *et al.*, 2010), isolation (Pedersen *et al.*, 2006), longevity (Vogel *et al.*, 2009) and aggression (Buczkowski *et al.*, 2004). In *Technomyrmex albipes*, between-supercolony variance in sex allocation exceeds within-supercolony variance (Tsuji & Yamauchi, 1994). Besides plasticity, variation may arise from genetic drift and very strong constraints on gene flow between supercolonies (Jaquiéry *et al.*, 2005; Drescher *et al.*, 2010). An important next step will be revealing how much of this variation is indeed genetic and how such features covary with supercolony reproductive success.

α: Intensity of competition

Selection between groups, with the reduction of within-group selection, defines any ETI. Driving this selection, competition in paradigmatic Darwinian populations is close to being a zero-sum game (high α), such that an entity's gain in competitive success is compensated elsewhere in the population by a decline in the success of another (Godfrey-Smith, 2009).

α at the lower level (nest)

Nonsupercolonial nests are clearly in competition for survival. By definition, nests within supercolonies have congenial relations between one another, and in this sense, the familiar intense competition that characterizes ant nests is absent (Fig. 2). However, it is possible that, at a broad scale, large nest clusters of locally elevated relatedness are in competition, as a consequence of viscous gene flow within supercolonies (Chapuisat *et al.*, 1997; Helanterä, 2009; Holzer *et al.*, 2009).

The magnitude of α is shaped directly by common de-Darwinizers. One such mechanism is the evolution of policing, in which lower-level units pay to prevent defection by other units (Frank, 1995; Brandvain & Wade, 2007). Whether these policing processes – which include 'immune surveillance' against cancer (Pradeu, 2013) – have analogues in supercolonies remains an open question.

α at the higher level (supercolony)

α is likely to be negatively correlated with supercolony size (Pedersen *et al.*, 2006). High supercolonial turnover (34% p.a.) resulting from competition among genetically distinct small supercolonies in native *L. humile* has strengthened the case for intersupercolonial competition in certain taxa (Vogel *et al.*, 2009; Moffett, 2012a), especially in the native ranges of the invasive species. Such competition may, indeed, select against intrasupercolony instability arising from selfish behaviour at lower levels (Pedersen *et al.*, 2006), because it means the scales of cooperation and competition do not completely overlap. Such roles played by between-group competition in the evolution of group-level traits are currently under debate (e.g. Reeve & Hölldobler, 2007; Gardner & Grafen, 2009).

In taxa where supercolonies are often spatially separated (e.g. native *Myrmica*: van der Hammen *et al.*, 2002; *Formica*: this study), the extent to which supercolonies compete remains uncertain and perhaps can only be resolved with monitoring over the long term.

H: Fidelity of heredity

In Darwinian space, *H* refers to the similarity between parent and offspring phenotypes. Heritability estimates using parent–offspring regression describe a quantifiable aspect of *H* and would be valuable targets of future research on supercolonies.

H at the lower level (nest)

In nonsupercolonial systems, nest-level traits can show heritable variation (Gordon, 2013), although the extent of such heritability remains unknown. We believe that this is less likely within supercolonies, as parent–offspring relations are almost meaningless, due to the extraordinary movement of workers, queens and brood between multiple nests (e.g. Rosengren & Pamilo, 1983; Rosengren *et al.*, 1985, 1993; Holway & Case, 2000; Moffett, 2012a,b). Lack of strong genetic distinctions between nests has been observed in many cases (e.g. Ingram & Gordon, 2003; Jaquiéry *et al.*, 2005; Corin *et al.*, 2007; Vogel *et al.*, 2009), unless viewed across a very broad scale (Holzer *et al.*, 2009; Suhr *et al.*, 2009; Seppä *et al.*, 2012). Consequently, the populations of nests that comprise such supercolonies are not Darwinian populations: they cannot evolve by natural selection.

H at the higher level (supercolony)

We have alluded above to the possibility that supercolonies may show heritable variation in their internal cohesion (Pedersen *et al.*, 2006), but no cases of heritable variation in supercolonial fitness have yet been empirically documented. Although it is practically challenging, we encourage studies into heritable variation in populations of supercolonies, which may also aid in efforts to control invasive species (Lee, 2002).

Agnosticism about intrinsic fitness (S) and the fitness landscape (C)

In Wilson and Sober's (1989) seminal paper 'Reviving the Superorganism', superorganisms are defined by a combination of organism-like 'functional organization' and a valid claim to being a unit of selection. Although there is suggestive evidence that supercolonies partially satisfy the first of these conditions, the latter remains mysterious. This is largely because we cannot yet know the value of *S*, the extent to which fitness variation among supercolonies is dependent upon intrinsic factors. The high turnover in native *L. humile* (see above) may simply be a result of stochastic supercolony failure, and thus, *S* may be too low for supercolonies to function as selectable individuals. This is closely linked to *H*, and thus, we suggest that analysing the heritability of variation between supercolonies will help resolve this question.

Similarly, we choose to remain agnostic about *C*, the smoothness of the fitness landscape. A highly rugged fitness landscape thwarts paradigmatic evolution by natural selection. Studies into how differences in supercolony phenotype translate into differences in supercolony fitness will be challenging, although they may have implications for understanding the extraordinarily successful spread of invasive supercolonies around the world.

A significant next step will be situating supercolonies in Darwinian space using formal evolutionary measures. In particular, this will require determining the magnitudes of *S*, *H*, *V* and α – all of which are currently mysterious – to quantify the response to selection at different levels. The conceptual apparatus for such formal links is already well developed.

Conclusion

Theorists interested in individuality have often treated the issue in two separate ways (Haber, 2013). Firstly, a system can be a *metaphorical* individual, usefully analogous to – but not synonymous with – a homoeostatic, integrated organism. Many invocations of eusocial 'superorganisms' fall within this category, and it is easy to view the supercolony as a metaphorical organism. Strategic redistribution of resources and ants, for instance, is a sophisticated trait redolent of vertebrate closed circulation. Secondly, a system can be an *evolutionary* individual, a real cohesive whole inhabiting an evolving population amenable to traditional evolutionary analysis. We suggest that supercolonies inhabit a grey zone between these two concepts of the individual: it may be a useful heuristic to draw parallels between these collaborative populations and multicellular organisms, in accordance with the first concept, but a scenario of real individuation is not necessarily far-fetched.

Semi-integrated but lacking the reproductive traits associated with Darwinian individuality, supercolonies resemble asexually reproducing sponges (Fig. 2). We have argued that the nests within them have been conclusively de-Darwinized: they show extreme mosaicism and are not heritably distinct, rendering selection blind to between-nest variation. This does not mean that selection is extinguished at a matrilineal level: indeed, widespread matrilineal nepotism is likely to be held back only by constraints such as informational uncertainty (Helanterä, 2009). If these constraints were to be eroded, a resurgence of nest-level individuality might occur.

An important next step will be testing for differences in the Darwinian status of opposing forms of supercolonies. The current view is that the difference between invasive and native supercolonies is generally one of spatial scale, rather than a switch to a supercolonial state within a species during human-mediated invasions (Pedersen *et al.*, 2006). However, there may be significant differences between the two, and any supercolonial lineage arising from human activity may have had insufficient time for the divergence of supercolonial traits, especially if the response to selection is relatively mild at a supercolonial level.

In summary, for supercolonies to achieve individuation, there must be an increase in integration, largely through the loss of autonomy on the part of nests, an increase in reproductive specialization, an increase in the frequency of bottlenecks, high heritability of supercolony features and a clear link between such heritable variation and reproductive success. We now welcome empirical studies quantifying the extent to which these critical features of ETIs have been achieved in supercolonies, and encourage the study of high-level individuals in the construction of the biological hierarchy.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Methods for collection and analysis of empirical data reported in the main text.

Table S1 Worker size variation between nests (Kruskal–Wallis tests). Significant results are highlighted by asterisks.

Table S2 Among-nests variation in worker facial shape (MANCOVA).

Table S3 Analysis of among-nests variation in readiness to aggression (proportion of antennation events escalated to aggression) (Kruskal–Wallis tests).

Table S4 Analysis of variation in foraging preference (protein:carbohydrate ratio) in phylogenetically close supercolonies and nonsupercolonial populations (Fligner–Killeen tests).

Table S5 Analysis of variation in foraging effort (number of foragers at baits) (Fligner–Killeen tests).

Figure S1 A snapshot of sexual production in the supercolony.

Figure S2 Landmark assignment emphasized key features of facial anatomy.