Original Article

Consistency of animal social networks after disturbance

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Social networks encompass both individual and group phenotypes that have been shown to covary with fitness in several species. In order for network characters to be evolutionarily important, they must reliably reflect properties of an individual or groups of individuals; however, it is unknown whether network traits are consistently expressed at either level. To determine if measurable components of individual social network position were repeatable and if the network structure as a whole was consistent in *Bolitotherus cornutus* (the forked fungus beetle), we constructed 8 experimental populations. Half of the populations were disturbed between 2 observation periods. Two individual network metrics (strength and betweenness) were significantly repeatable across time in all treatments; a third (clustering coefficient) was not. At the network level, all 3 metrics changed more in undisturbed than disturbed networks. These findings suggest that individual network position can be a consistent property of individuals that is resilient to disturbance and could experience selection in a predictable fashion. However, group network structure seems to change over time unless reset by disturbance.

Key words: **Bolitotherus cornutus, disturbance, individual differences, repeatability, selection, social networks.**

INTRODUCTION

Social networks describe the complex structure of interactions within groups of individuals (Wasserman and Faust 1994; Croft et al. 2011). An individual's position in a network has been shown to affect its access to information and susceptibility to disease and parasites (reviewed in Whitehead 1997; Krause et al. 2007; Newman 2006; Croft et al. 2008; Wey et al. 2008). Individual fitness has also been shown to covary with position in a network in a number of systems (Silk et al. 2003; McDonald 2007; Silk et al. 2009; Ryder et al. 2009; Oh and Badyaev 2010; Formica et al. 2012; Wey and Blumstein 2012; Brent et al. 2013), suggesting that individual network position might be adaptive (Gordon 1996; Royle et al. 2012; Pinter-Wollman et al. 2013). Because the structure of networks as a whole can mediate the flow of information and disease, the network itself might affect the fitness of its participants and thereby the mean fitness of the population, raising the possibility that network structure can influence multilevel selection. However, it remains unclear whether individual or group network properties are expressed with the consistency required for social networks to have evolutionary potential.

Repeatability is a fundamental requisite for the evolution of behavioral traits because it addresses both the variance among individuals and individual consistency (Bell et al. 2009). In order for a trait to experience natural selection, there must be a covariance between that trait and fitness (Lande and Arnold 1983; Brodie et al. 1995). If individuals express different trait values across time, then no predictable relationship between the trait and individual fitness is likely to emerge (Boake 1989; Brodie 1993; Brodie and Russell 1999). Recent focus on individual differences and animal personality has revealed that behaviors are surprisingly repeatable traits (Sinn et al. 2006; Smith and Blumstein 2008; Bell et al. 2009; Blumstein et al. 2012). However, it is difficult to extrapolate this general finding to network traits because an individual's position within a social network is an interacting phenotype whose expression is influenced by the individual's own phenotype, as well as the properties of its immediate social partners (Moore et al. 1997), and the emergent structure of the network at large.

The influence from social partners could impact the repeatability of social network position to varying degrees. We might expect individuals to retain similar positions in a network across time if their behavioral traits such as boldness, exploration, and activity are reliably expressed (Croft et al. 2005; Pike et al. 2008; Croft et al. 2009; Krause et al. 2010; Schürch et al. 2010; Webster and Ward 2011; Blumstein et al. 2012), in which case network traits

would present predictable phenotypes on which selection could act (e.g., Barocas et al. 2011; Brent et al. 2013; Wey et al. 2013; Jacoby et al. 2014; Aplin et al. 2015; Frumkin et al. 2016). Alternatively, higher-level interactions among many individuals and nonlinear effects thereof might substantially shape the structure of networks, leaving individual behaviors with little impact on an individual's position in a network across time. It is also possible social networks reflect more stochastic patterns of interactions, whereby individuals could be more or less randomly located within a network each time it is characterized. In these 2 latter cases, individual network position would not be repeatable and therefore unlikely to experience selection and to evolve.

An individual's position in a network is described by a variety of metrics that capture the pattern of connections among individuals and subsets of the group (Wasserman and Faust 1994; Whitehead 2008; Croft et al. 2011). At one end of a spectrum, some metrics characterize immediate interactions that might be heavily influenced by the properties of the focal individual (e.g., "strength," a measure of how often an individual interacts with other individuals). At the other end are metrics that describe secondary and tertiary patterns that require complete information of the entire network structure and the connections among other individuals (e.g., "betweenness," a measure of how many network paths pass through an individual; Croft et al. 2008). These higher-order metrics depend heavily on interactions among conspecifics several degrees removed from a focal individual, and might therefore be expected to be less repeatable at the individual level.

Just as the evolutionary potential of individual traits requires repeatability, multilevel selection can only be effective when group-level characters are reliably expressed. Networks can also describe emergent features of the group as a whole that could affect the fitness of the individuals within the network, and thereby experience multilevel selection. These group-level features are typically measured as the average of individual metrics within the network (Croft et al. 2008). For example, mean betweenness of a network is thought to impact the rate that information or disease can flow through a population (Ortiz-Pelaez et al. 2006; Salathé et al. 2010). Group-level network traits have been associated with individual fitness (Royle et al. 2012) and might therefore be expected to evolve through multilevel selection processes (sensu Okasha 2004b, 2006).

To address the evolutionary potential of social network characters, we evaluated their repeatability of expression at the individual level and the consistency of expression at the group level. We first asked if position in a network is a repeatable trait of an individual. We hypothesized that the metrics most closely associated with primary interactions (e.g., strength) would be repeatable and those that involve higher-order interactions among partners (e.g., betweenness and clustering) would not be repeatable. We then examined individual-level repeatability and group-level consistency in the presence of a disturbance, hypothesizing that a disturbance in which all individuals were temporarily isolated would eliminate the repeatability of individual network position. We also asked if the overall structure of networks was consistent over time, and resilient to disturbance. We hypothesized that networks reformed after a disturbance would deviate from their original structure, whereas networks left in an undisturbed treatment would express a consistent structure across time.

METHODS

Study system

Bolitotherus cornutus is a beetle that occurs in forests throughout eastern North America (Liles 1956). The species depends at all life stages on polypore shelf fungi that infect dead trees; eggs are laid on the fruiting bodies of the fungus ("brackets"), larvae feed on and develop within the brackets, and adults forage on the bracket surface (Pace 1967). Virtually, all social interactions among adults occur on or near the surface of these brackets (Conner 1988; Formica et al. 2011). Male *B. cornutus* have horns that are used in combat over mates and are under strong sexual and social selection in low-density populations (Conner 1988, 1989; Formica et al. 2011). Males attack other males during courtship, mate guarding, and Supplementary Figure S1 occasionally during solitary feeding events, but rarely attack females. Losers of these bouts are occasionally thrown from the fungal brackets to the forest floor below (Formica V, personal observation). Females lack horns and have never been observed initiating aggressive physical interactions, though they also use the surface of fungal brackets for oviposition and foraging (Pace 1967).

Social interactions in this species do not require physical contact. *Bolitotherus cornutus* often orient and wave their antennae toward conspecifics when they come within a few body lengths of one another, Supplementary Figure S2 suggesting chemical or vibrational communication is important. Physical proximity is the first stage in all intersexual and intrasexual interactions, and leads directly to the agonistic and courtship interactions described above. The majority of *B. cornutus* behavior is observed at night, although they are active throughout the day. Home ranges of individuals within a log vary dramatically with some individuals remaining on or near a single bracket most of the season, whereas others range across the entire log (Formica et al. 2010). Previous work in a natural population, using a male-only social network based on physical proximity, found 2 individual position metrics, strength and clustering coefficient (see below), covaried with male mating success (Formica et al. 2012).

Experimental populations

Experimental arenas were constructed in plastic containers measuring 0.6 m × 1.8 m. Plaster of Paris (DAP Products, Baltimore, MD) was poured into each arena to a depth of approximately 3 cm to retain moisture, and hardwood mulch was pressed into the surface of the plaster to mimic the surface of a log. Seven brackets of wood-decaying shelf fungus (*Ganoderma applanatum*) were embedded perpendicular to the plaster at regularly spaced intervals. Bracket senescence was scored on a qualitative scale according to bracket size and the amount of live hymenium present. Each arena received one bracket from each senescence category and all brackets from the same category were assigned to corresponding spatial locations in each of the 8 arenas. Arenas were housed in a room with controlled temperature (~21 °C), humidity (70% in the air and ~100% at the surface of the brackets), and photoperiod (8:16 h dark:light) to approximate local conditions at Mountain Lake Biological Station (Giles County, VA) in early May, when the beetles are most active in the wild (Ludwig 2008). The light cycle was reversed from ambient conditions to allow for easier observation and to ensure that the peak period of behavior of the nocturnal *B. cornutus* was the most frequently observed. During the dark portion of the beetle photoperiod, dim red lights were left on to facilitate observer movement in the dark.

The beetles and fungus used in this experiment were collected from a total of 32 wild populations in Giles and Craig Counties, VA, between 17 May and 30 May 2010. All individuals were imaged on a flatbed scanner (Epson Perfection V600 Photo, Suwa, Nagano, Japan) and their elytra (the hardened forewings) measured in ImageJ (Abramoff et al. 2004). Each experimental population was stocked with 15 male and 15 female *B. cornutus*, for a total of 30 beetles per arena. In the wild, patches of fungus can contain from 1

to over 200 individuals. Our design resulted in a density of 2 males per bracket, which is a high density for the local meta-population (Formica et al. 2011) but within the natural range observed in this species (Conner 1989). To minimize social familiarity among the individuals, no more than 2 beetles of each sex from the same population of origin were assigned to the same experimental group. All 8 experimental groups had the same mean and variance in body size (elytra length) for both males and females. Unique identification tags were affixed to the elytra of each beetle with light-cured acrylic (Tuffleye Wet-A-Hook Technologies, San Antonio, TX), and a small dot of the same acrylic was applied to the elytra to prevent flight. A dot of glow-in-the-dark enamel (Valspar, Chicago, IL) was placed on the elytra of each beetle to enable observers to locate it in the dark.

At the start the experiment, all individuals were released at the center of their respective arenas. After an initial acclimation period of 24 h, each arena was scanned every day at 0900, 1300, 1600, and 1900 with ultraviolet and red light. Arenas were scanned in a haphazard order each observation period. During a scan, the location, behavior, and social partners of all visible beetles were recorded. Not all beetles could be located during every observation because beetles could hide under pieces of mulch or in cracks in the plaster; however, at the end of the experiment, we were able to locate all beetles to ensure none had escaped. Social partners were defined as any beetle within 3 cm (i.e., approximately 2 body lengths) of the focal beetle.

We used spatial proximity to delineate social interactions for several reasons. Spatial proximity is a common method for constructing social networks (e.g., Wey et al. 2013; Aplin et al. 2015; Leu et al. 2016). Position within a spatial proximity network has previously been correlated with copulation success in male-only networks of *B. cornutus*, indicating that patterns of social interaction important to fitness may be captured in spatial proximity networks in this species (Formica et al. 2012). Second, personal observation of *B. cornutus* behavior suggests that touching is not necessary for the transfer of social information. For example, beetles reorient when others approach before contact, males will occasionally turn and increase speed toward a female before courting her, and males have been observed charging other males to initiate combat in video recordings. Lastly, many beetle species (including *B. cornutus*) have complex volatile compounds (Holliday et al. 2009) that may allow beetles to gain social information without contact (e.g., Obengofori 1991; Wertheim et al. 2005).

Four of the 8 experimental populations were assigned to a disturbance treatment, and 4 left undisturbed. During the first 12-day observation period, all groups were treated identically. At the end of the observation period, individuals in the "undisturbed" populations remained in the same arena as before and were allowed to interact with each other freely throughout the duration of the experiment. Individuals in the "disturbed" populations were captured and removed to individual holding chambers where they could not interact with other beetles. After 4 days of isolation, all individuals from the disturbed treatment were reunited with the same individuals from their respective populations in the initial observation period at the center of a different experimental arena. Groups of beetles were moved to new arenas to disrupt any spatial component of a group's previous social network, and to control for arena effects. Twenty-four hours after the disturbed beetles were returned to the arenas, observation of both disturbed and undisturbed populations resumed for another 12 days. During the course of the experiment, 2 males died early in the first time period, one in an undisturbed population and one in a disturbed population; both individuals were excluded from analysis.

Social network metrics

We built weighted and symmetrical networks for each of the 8 populations separately for the 2 observation periods in the R package tnet (Opsahl 2009) using the simple ratio index (SRI) as our association index. The SRI is calculated as $\dfrac{X}{(O-D)}$ where X equals the number of times 2 individuals (dyads) interacted, O is the total number of observation periods, and D is the number of observations where neither individual in the dyad is observed (Ginsberg and Young 1992; Croft et al. 2008). The SRI results in a value between 0 and 1 for all pairs of individuals in each artificial population. A value of 1 would indicate that every time either individual was observed they were found associating with the other, and a zero would indicate that the individuals were never seen associating with each other. A total of 16 networks were constructed (4 undisturbed and 4 disturbed in period 1, and 4 undisturbed and 4 disturbed in period 2).

Three weighted network metrics were calculated for each individual in each network—strength (alpha = 0.5), betweenness, and local clustering coefficient—using formulas reported in the tnet documentation (Opsahl 2009). These measures were chosen because they are common measures of position in weighted networks (Farine and Whitehead 2015) that measure different aspects of node-level position. Each has been previously examined in this study species (Formica et al. 2010, 2012). Strength and betweenness are measures of network centrality and describe how important a node is to the structure of the network; nodes that have many connections or connect different parts of a graph are often said to be central. Strength is defined as an individual's number of unique social partners, weighted by the frequency of their social interactions. When the tuning factor (alpha) is set to 0.5 (as it is here), repeated interactions among the same pairs of beetles are discounted by a half (Opsahl 2009). Betweenness is defined as the number of simplest paths between all individuals in the network that pass through the focal individual (i.e., the paths from one individual to another through the shortest geodesic routes with the greatest edge weight) (Wasserman and Faust 1994; Opsahl 2009). Clustering coefficient is a measure of cliquishness, quantified as the proportion of and frequency with which an individual's social partners interact with each other (Croft et al. 2008). Beetles with one or zero partners have an undefined value for clustering coefficient and were excluded from models examining clustering coefficient.

Multilevel selection requires repeatability of traits at the population level, just as individual selection requires repeatability among individuals. For network traits to be repeatable at the group level, variance among populations must be greater than variance within populations for a particular metric (e.g., strength). Our experimental design sought to minimize differences among the artificial populations (similar sex ratios, distributions of body size, population densities, and spatial arrangement of resources), thereby minimizing sources of among-group variance in network-level traits. Repeatabilities of network-level traits based on these manipulations would be difficult to interpret, so instead we tested for population-level consistency using generalized linear mixed models (GLMMs) that analyzed our experiment as a split-plot design (see Population-level consistency for further details) (Altman and Krzywinski 2015). These models allowed us to estimate the effect of time

period-by-treatment interactions at the population level, which reveal whether network-level measures change differently over time in different treatments.

Statistical analyses

Individual-level repeatability

We calculated repeatability for each of the individual network metrics: strength, betweenness, and clustering coefficient. Repeatability describes the proportion of total variance in a trait that is attributable to differences among individuals (Falconer and Mackay 1996) and is typically measured as the intraclass correlation coefficient (ICC) (Bell et al. 2009). We estimated ICC between first and second observation periods from a nested Anova (Whitlock and Schluter 2014). This analysis was run separately for each metric and for the undisturbed and disturbed treatments, and included population and individual nested within population as random effects. These analyses resulted in a measure of repeatability for each metric in the undisturbed populations and another in the disturbed populations (Table 1).

Significance testing in analyses of individual network position is complicated because each individual's position is (by definition) directly influenced by the position of its social partners (a violation of the assumption of independence), and metric distributions are often non-normal. However, because almost all networks generated by animals are nonrandom in structure, randomly generated networks are not the appropriate null. We therefore employed permutation tests to determine whether the ICCs for each metric in each of the 2 treatments were more extreme than ICCs calculated from permuted networks. The edges (including those with weights of zero) from the observed networks were pooled across the 4 replicates of each period and treatment combination and in each permutation, each edge was randomly assigned without replacement to connect 2 individuals. This approach shuffles interactions among individuals while holding the number of individuals (nodes) and the number and weight of their interactions (edges) constant. For each permutation, new networks were constructed, network metrics measured, and ICC values calculated. We performed 10 000 permutations for each test. This method retains the distribution of edge weights within the statistical group being considered and allows us to compare individuals in different populations in the same model. Two-tailed P values were calculated as the proportion of ICCs from the permuted networks that were more extreme than the observed values (Pratt and Gibbons 2012). The permutation tests were designed to test for significant repeatability within each treatment but cannot be used to draw comparisons of ICCs across treatments or metrics.

Population-level consistency

To calculate estimates of consistency at the group level and to determine if disturbance affects consistency, we conducted

GLMMs with each network metric as the dependent variable; treatment (disturbed or undisturbed), observation period (time 1 or 2), the treatment-by-period interaction, sex, body size, and activity (number of times observed on the surface of the artificial log or fungal brackets) were used as independent variables. Because individuals were observed twice (2 time periods), we included individual ID as a random effect. Population was also included as a random effect. This design is analogous to a split-plot repeated measures design, which is sensitive to detecting interactions across factors (Altman and Krzywinski 2015). A significant effect of period in this model indicates a change between observation periods (i.e., a lack of consistency), an effect of treatment indicates a difference in the network metrics between the disturbance treatments, and the treatment-by-period interaction tests whether the disturbance affected the consistency of the networks at the population level. Sex, body size, and activity level were included as covariates in the model to control for properties of individuals that have the potential to affect network structure above and beyond the disturbance treatment.

Significance testing of the fixed effects in these models of network-level consistency employed a similar permutation method as described above for the individual repeatability analysis. However, for the network-level consistency models, we permuted edges across individuals, populations, time periods, and treatments, allowing us to explicitly test hypotheses concerning these variables. P values were calculated as the proportion of permuted regression coefficients that were more extreme than the observed coefficients. We conducted all of the statistical tests and permutations in R version 3.1.1 (R Development Core Team 2011), and model coefficients were calculated using the lme4 package version 1.1-7 (Bates et al. 2014).

To report means and error for treatment groups and sexes (e.g., Figure 2), we calculated least squares means using the R package lsmeans (version 2.17; Lenth 2016). Ninety-five percent confidence intervals (CIs) around the least squares means were done with 10 000 bootstraps of the general linear model using the boot package (version 1.2-11; Davison and Hinkley 1997; Canty and Ripley 2016) and lme4 (Bates et al. 2014).

RESULTS

Each of the 8 artificial populations formed well-connected networks with a single interconnected component during each observation period (Figure 1). Across all populations, individuals interacted with a mean of 11.4 ± 4.2 standard deviation (SD) social partners during each 12-day time period. Only 3 individuals out of the 238 surviving individuals had zero social partners in a single time period and those 3 individuals became connected (had at least 1 social partner) during the second time period. Across all treatments and period, strength was correlated with betweenness and clustering coefficient, but betweenness and clustering coefficient were not significantly correlated (Table 1).

Repeatabilities were calculated separately for each metric and each treatment for a total of 6 separate permutation tests. At the individual level, strength and betweenness were significantly repeatable in both the disturbed and undisturbed treatment groups (Table 2). Clustering coefficient, however, was not repeatable in either treatment. The zero repeatability for clustering coefficient indicates that the between individual variability is not large enough to necessitate the inclusion of the random effect (individual) in the model (Bates et al. 2014; Table 2).

Table 1

Summary statistics for social network metrics across all periods, treatments, and both sexes

	Mean ± SD	Strength	Betweenness
Strength	2.44 ± 0.95	—	—
Betweenness	17.11 ± 18.89	**0.67 (<0.001)**	—
Clustering coefficient	0.49 ± 0.13	**0.14 (0.003)**	-0.07 (0.09)

The two right most columns represent Spearman's rho values (P values) for pairwise correlations.
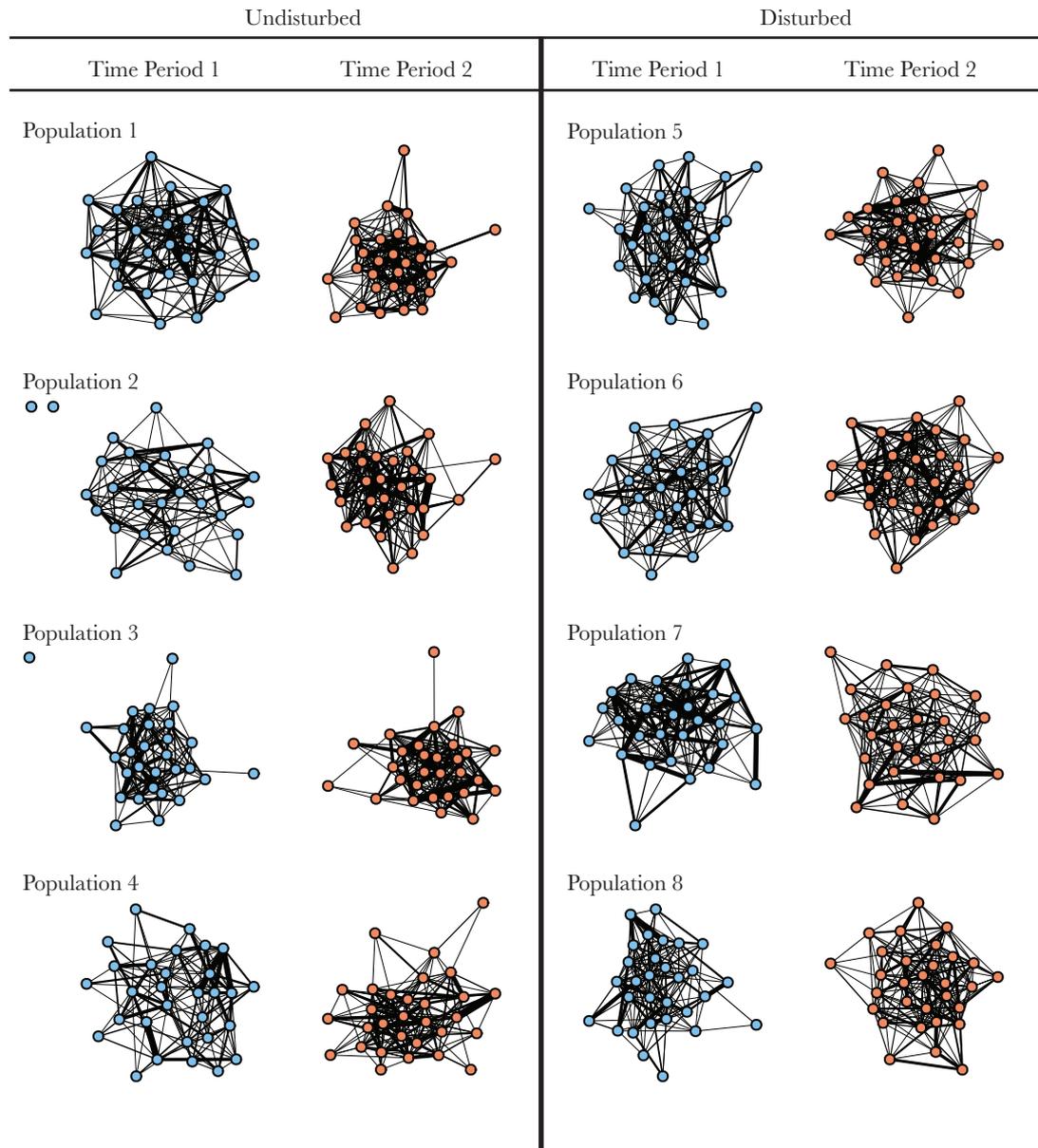
Figure 1

Network visualizations of all 8 artificial populations in both treatments for the first and second time periods. Circles (nodes) represent individuals and lines connecting the circles (edges) represent observed social interactions, the thickness of which indicates the number of interactions between the social partners. Populations 2 and 3 had a few individuals that were not seen interacting with any other individuals and are represented by unconnected nodes. The visualizations were constructed using the Fuchterman–Reingold method in iGraph for R (Csardi and Nepusz 2006; Fruchterman and Reingold 1991), which essentially pulls nodes that are highly connected closer together. Networks in the undisturbed treatment in time period 2 (second column) are denser and individuals are more broadly connected across the population's network than all of the other networks.

In the network-level analysis, 2 of the 3 network metrics exhibited differences between the sexes. Females had significantly higher centrality in the network, as measured by strength and betweenness, than males (least squares means [95% bootstrapped CIs] strength: males = 2.24 [2.16–2.33], females = 2.64 [2.55–2.71]; betweenness: males = 13.09 [11.36–15.18], females = 21.10 [19.12–22.86]; Table 2). These results suggest that females interacted more often or with more individuals, positioning them along more paths to others in the network than males. Males and females did not differ in their individual cliquishness (clustering coefficient: males = 0.48 [0.46–0.50], females = 0.49 [0.48–0.51]; Table 3). Activity was significantly associated with all 3 metrics (Table 3); individuals who

were more active had more and stronger connections were more centrally located to the paths between individuals and were less cliquish. Body size was not related to any of the network metrics.

Treatment-by-period interactions for all 3 network metrics indicated that disturbance had a significant effect on the consistency of overall network structure (Table 3). Strength, betweenness, and clustering coefficient all increased from the first to the second observation period in the undisturbed populations, but not in the disturbed populations (Figure 2). Although some measures of group network structure changed over time in undisturbed groups, disturbance seemed to act as a reset that returned group strength and clustering coefficient near the initial values observed in the first period.

DISCUSSION

We found strong evidence for the repeatability of some social network traits at the individual level and consistency of some network traits at the group level. The disturbance did not reduce the repeatability of individual-level network traits, but instead caused group-level network structure to remain similar over time. Social network characters therefore may be subject to both individual-level and multilevel phenotypic selection. Whether this translates to evolvability ultimately depends on whether such traits are heritable across generations, which remains to be demonstrated.

Repeatability of individual network position

Contrary to the expectation that interacting phenotypes—such as social network position—might have lower repeatabilities than other behavioral traits because their expression depends on social partners (Boake 1989; Bell et al. 2009), our results demonstrate that at least some measures of network centrality (strength and betweenness) were significantly repeatable. This result suggests that they may be able to experience selection. These significant repeatabilities indicate that individuals in the first time period who interact more often (high strength), and with social partners from different parts of the population (high betweenness) continue to do so in the second time period. The magnitude of repeatability for both strength and betweenness was comparable with those reported in a recent meta-analysis for other behavioral traits such as activity (mean $r = 0.24 \pm 0.18$ SD), mate preference (0.25 ± 0.28), affiliation (0.29 ± 0.09), and parental care (0.29 ± 0.28) (Bell et al. 2009). Our experimental design may have been especially likely to find repeatability in social network traits, relative to a design that shuffled

individuals among social groups. Therefore, our estimates might be thought of as upper bounds on the repeatability of these traits.

We expected that primary network measures such as strength would be more heavily influenced by properties of focal individuals and therefore would be more repeatable. However, we also found that a more composite measure of centrality, betweenness—which is influenced by social interactions in which the focal individual does not participate—was similarly repeatable. The repeatability of both strength and betweenness combined with their strong correlation (Spearman's $\rho = 0.67$; Table 1) suggests that similar underlying behaviors of individuals may lead to stable expression of centrality to the network. Our findings in a beetle species add to a growing body of work in vertebrates demonstrating that individual centrality (measured by metrics such as strength, betweenness, and degree) in social networks are repeatable extended-phenotypes in variety of taxa (Brent et al. 2013; Jacoby et al. 2014; Aplin et al. 2015; Frumkin et al. 2016; Leu et al. 2016).

Measures of cliquishness capture patterns of association that depend on interactions between more than 2 individuals. For this reason, we were not surprised that clustering coefficient exhibited no measurable repeatability in our experiment. Contrary to our findings, Jacoby et al. (2014) found clustering coefficient to be a significantly repeatable network trait in juvenile sharks, suggesting that there may be species differences in which network traits are repeatable.

Network-level consistency

Network-level characteristics showed surprising similarity when groups of beetles were allowed to reform networks after a period of isolation. Although there were small changes for clustering coefficient and strength, all mean network-level metrics changed very little between the 2 observation periods in groups of beetles that experienced the disturbance. The disturbance appeared to "reset" social structure in our experimental populations. These results suggest that there are emergent network properties of collections of individuals that are consistently expressed, even when the groups reestablish connections after isolation. In contrast, the networks of populations that were left undisturbed exhibited significant changes in structure, becoming more centralized (higher mean strength and higher mean betweenness) and more cliquish (higher mean clustering coefficient) (Figures 1 and 2). Although the networks in the undisturbed treatment changed from a loose to a dense architecture between the time periods, there was little change in the overall architecture in the disturbed treatment (significant treatment-by-period interactions, Table 3). Our disturbance included both solitary confinement and a transition to new location, so we cannot

Table 2

Intraclass correlation coefficients (2-tailed *P* values are in parentheses)[a] for each network metric, presented for both treatments

	Undisturbed (*P* value)	Disturbed (*P* value)
Strength	**0.19 (<0.001)**	**0.14 (0.037)**
Betweenness	**0.25 (<0.001)**	**0.29 (0.003)**
Clustering coefficient	0 (1)	<0.001 (0.304)

[a]*P* values are reported as the proportion of ICC values less extreme than the observed value across 10 000 permutations. Each social network metric and treatment was run in separate permutation models. The zero value for clustering coefficient in the undisturbed treatment indicates that the between individual variability is not large enough to necessitate the inclusion of the random effect (individual) in the model.

Table 3

Observed, unstandardized regression coefficients from GLMMs (*P* values are in parentheses)[a] for each network metric from 2-tailed permutation tests

Factor[b]	Strength	Betweenness	Clustering coefficient
Treatment	**0.09 (0.006)**	**1.61 (<0.001)**	0.01 (0.39)
Period	**−0.27 (<0.001)**	−0.77 (0.07)	**−0.03 (<0.001)**
Treatment × period	**−0.14 (0.001)**	**−0.53 (<0.001)**	**−0.03 (<0.001)**
Sex	**0.19 (<0.001)**	**3.99 (<0.001)**	0.01 (0.09)
Body size	−0.08 (0.24)	0.46 (0.74)	−0.01 (0.13)
Activity	**0.07 (<0.001)**	**0.99 (<0.001)**	**−0.002 (<0.001)**

[a]*P* values are reported as the proportion of model coefficients more extreme than the observed value across 10 000 permutations. Each social network metric and treatment was run in separate permutation model/GLMM.
[b]Factor reference categories were treatment: undisturbed, period: period 1, sex: female.
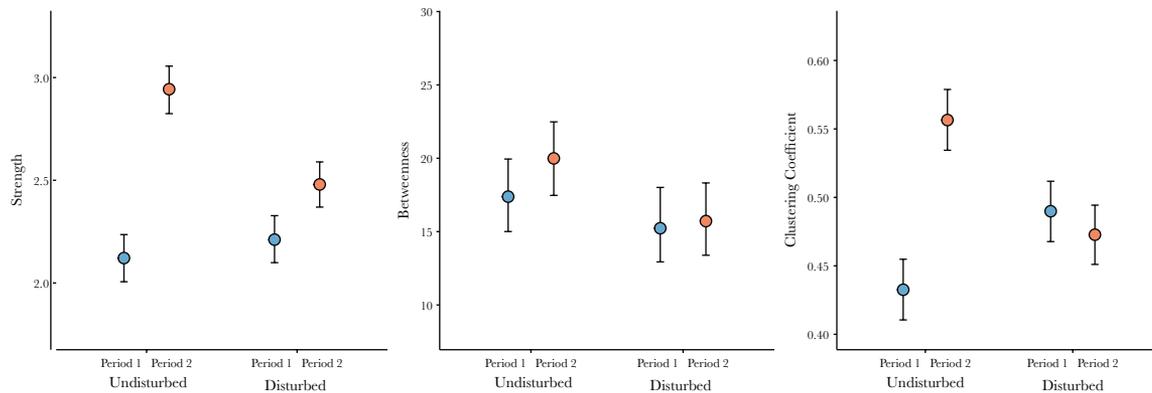
Figure 2
Least squared means from GLMMs for network-level traits. Ninety-five percent confidence intervals (error bars) were calculated with 10 000 bootstraps from the observed data. Statistical details are reported in Table 3.

disentangle the effects of those 2 manipulations on network consistency. It may be that solitary confinement resets the social patterns that change in the undisturbed populations, or that exploring new environments generates similar patterns each time.

The repeatability of an individual-level character did not predict its consistency at the group level. Although strength and betweenness were repeatable within individuals in both treatments, the mean strength and mean betweenness of the network both increased through time in the undisturbed treatment (Figures 1 and 2). This finding suggests that although all individuals are increasing their number of partners and interactions with them, their relative differences remain the same. Conversely, the overall cliquishness of a network did not change in disturbed networks (the other treatment), even though the clustering coefficient was not detectably repeatable at the individual level (Table 2). In other words, even though some individuals became more cliquish and others became less cliquish between the time periods, the populations maintained the same mean level of cliquishness across time in the disturbed treatment (Figure 2, far right).

Our group-level analysis also revealed that although there was no relationship between body size and any of the metrics, females were significantly more central to the networks (higher strength and betweenness) than males. Further experiments are needed before we can determine the cause of the sex differences in the centrality, but we hypothesize that females may experience more social contact with individuals because they are being courted by many different males, whereas some males may have limited social contact with each other and females due to indirect and direct competition with a few, aggressive males.

Practical considerations

Our results underscore the sensitivity of network characteristics to the specific conditions in which they are assayed. First, removing individuals from an interacting population (even for a few days) may change the structure of the social network, potentially resetting the network to a previous state. In mark–recapture studies, one or a few individuals are often captured and temporarily removed from the population. It is unclear what impact this would have on network structure, but our results suggest that caution should be taken in the removal of individuals when the structure of the social network is important to the study. Second, groups of individuals housed together for long periods, or observed over long durations in the wild, may exhibit dynamic network structure. Many investigators

of networks in wild animals tend to aggregate social observation data over long periods of time when constructing social networks. Constructing social networks over such long periods may obscure biologically relevant information about the dynamic structure of networks. Because our study only examined the artificial populations for 2 short time periods (2 weeks each period), we cannot determine whether new networks go through a burn-in period and reach equilibrium, or are constantly in a state of flux. However, it is clear from our observations that networks sampled over short periods of time may only be snapshots of a dynamic process.

Evolutionary implications

The repeatable expression of betweenness and strength is one necessary condition for these network characters to evolve because it directly impacts the potential for individual-level phenotypic selection. Clustering coefficient does not meet this requirement in these populations and is unlikely to have a predictable relationship with individual fitness. This latter result is striking because clustering coefficient is the only metric that has been shown to covary with fitness in male–male networks in wild *B. cornutus* (Formica et al. 2012). The lack of repeatability indicates that the previously observed relationship between cliquishness and male-mating success might not be replicable across populations or time. However, caution must be taken when interpreting repeatabilities from controlled laboratory studies in the context of wild populations (Bell et al. 2009) and it remains to be seen whether the repeatabilities we measured in this experiment accurately reflect patterns of variance in the field.

Disturbances in the wild such as those that might arise from environmental fluctuations, migration, and mortality could contribute to the stable expression of network-level characters, and thereby the potential for multilevel selection. Multilevel selection occurs when group-level characteristics covary with the fitness of individuals within them (Heisler and Damuth 1987; Okasha 2004a; Formica et al. 2011; Goodnight 2013). Just as with individual selection, reliable expression of group-level characters is necessary for multilevel selection to occur. Our results suggest that the long-term stability of network-level characters may depend on the frequency and impact of disturbances in the wild. However, our disturbance was entirely artificial (solitary confinement and introduction to a new space), and so it remains unknown how naturally occurring disturbances affect temporal consistency of network-level traits. Additionally, our experiment examined relatively short periods of time for network formation (12 days each period), and group-level consistency may have more complicated

dynamics over longer periods of time. The effect of disturbance on network traits might also differ across species. For example, species with higher cognitive function or high levels of individual recognition might be more resilient to extrinsic disturbances, thereby letting the dynamic nature of networks progress without resetting.

Ultimately, it is the repeatability of network-level traits relative to other networks within a population that determines the potential for multilevel selection (Okasha 2004b, 2006). Because our experiment was designed to minimize variance among the artificially constructed populations, our data do not address the true network-level repeatability in the wild. The next step in understanding the role of multilevel selection in the evolution of social networks should be to examine the variance among wild populations in group-level network traits and their covariance with individual fitness using a contextual analysis paradigm (Heisler and Damuth 1987; Okasha 2004a; Formica et al. 2011; Goodnight 2013).

Although our results demonstrate that phenotypic selection might operate on network characters, evolutionary response requires their transmission across generations in a predictable fashion (i.e., heritability). Heritability of some network and sociality characters has been demonstrated in humans, macaques, and marmots (Fowler et al. 2009; Lea et al. 2010; Brent et al. 2013) but has only begun to be explored in animal networks. Traditional methods of estimating heritability may be difficult to apply to network traits because they are only expressed in interactions with conspecifics. Genetic variance for network traits, if it exists at all, is likely to be influenced by the genes of focal individuals and the genes of their social partner, as well as interactions between them. Therefore, understanding the evolutionary potential of individual network position and group-level network properties will require an approach that incorporates direct and indirect genetic effects (Moore et al. 1997; Wolf et al. 1999; McGlothlin et al. 2010).

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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