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Report

Cryptic Plasticity Underlies a Major Evolutionary Transition

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Summary

The origin of eusociality is often regarded as a change of macroevolutionary proportions [1, 2]. Its hallmark is a reproductive division of labor between the members of a society: some individuals ("helpers" or "workers") forfeit their own reproduction to rear offspring of others ("queens"). In the Hymenoptera (ants, bees, wasps), there have been many transitions in both directions between solitary nesting and sociality [2–5]. How have such transitions occurred? One possibility is that multiple transitions represent repeated evolutionary gains and losses of the traits underpinning sociality. A second possibility, however, is that once sociality has evolved, subsequent transitions represent selection at just one or a small number of loci controlling developmental switches between preexisting alternative phenotypes [2, 6]. We might then expect transitional populations that can express either sociality or solitary nesting, depending on environmental conditions. Here, we use field transplants to directly induce transitions in British and Irish populations of the sweat bee *Halictus rubicundus*. Individual variation in social phenotype was linked to time available for offspring production, and to the genetic benefits of sociality, suggesting that helping was not simply misplaced parental care [7]. We thereby demonstrate that sociality itself can be truly plastic in a hymenopteran.

Results and Discussion

Sweat bees (Halictinae) are unusually valuable for elucidating social transitions. Unlike other hymenopteran lineages, closely related social and solitary sweat bees are extant [5], perhaps because halictine sociality evolved relatively recently [3]. There are also socially polymorphic taxa such as *Halictus rubicundus* (Figure 1D), where females in some populations form social groups whereas those in other populations nest solitarily [2–4, 8, 9]. Such taxa provide ideal material to investigate the underlying basis of variation in social phenotype [4, 8–11]. In spring, each *Halictus rubicundus* foundress digs a separate nest burrow where she rears a first brood (B1) of approximately six offspring. In solitary populations, mated B1 females overwinter before restarting the cycle. In social populations, however, some B1 females (referred to below as "B1 provisioners") become helpers, which forage to provision a second brood (B2) of overwintering offspring [4, 8, 12].

In Britain and Ireland, northern or high-altitude populations of *H. rubicundus* are solitary, whereas southern, low-altitude populations are social ([13]; unpublished data). To test for social plasticity, we first transplanted foundresses from a source site where native nests are social (Wicklow) to a destination site where natives are solitary (Peebles) (Figure 1A). The result was clear cut. All 79 nests of transplanted foundresses were solitary at the destination site: B1 females entered hibernation and did not provision their natal nests. In the same year, by contrast, B1 females provisioned a second brood in >90% of 33 nests monitored at the Wicklow source site (Figure 1B). Plasticity was not limited to bees from a single site: a further seven nests established by bees transplanted from a second social site (Cambridge) were also solitary at the Peebles destination site. However, transplantation per se did not induce a switch of phenotype: 57 nests established by foundresses transplanted from a solitary control site (Penrith) retained solitary nesting at the same destination site (Peebles).

Bees from social sites may be predisposed to exhibit plasticity. Two phenotypes are routinely expressed at such sites, albeit at different times of year: foundresses are solitary until B1 offspring mature. Bees from solitary sites, however, seem the least likely to exhibit plasticity, because sociality is never expressed at such sites. We therefore tested for plasticity in the reverse direction, by relocating foundresses from a site where nests are solitary (Belfast) to a lowland southern site where sociality would be expected (Sussex) (Figure 1A). We have never observed sociality in Belfast *H. rubicundus*, despite monitoring >100 nests in each of four consecutive years (2006–2009). Nevertheless, nearly half (46%) of 28 nests initiated by Belfast foundresses at Sussex became social (Figure 1C), with 3.5 ± 0.2 B1 provisioners per nest. Although B1 provisioners were the same size as foundresses from their own nests (t test, $p = 0.97$), we confirmed that sociality was not simply nest-sharing among equally reproductive females. Genotyping of adults and immature B2 offspring from social nests instead revealed a clear reproductive division of labor: reproduction was strongly skewed toward a single female (queen) in each nest (Figure 1E: mean skew using the recommended B-index = 0.47, $p < 0.0001$ compared with random expectation [14]). The foundress was still producing B2 offspring at a minority of the social nests, but had been replaced as queen by a B1 female at the others (Figure 1E). Behavioral observations combined with the genotyping showed that replacement queens were the oldest B1 females at their nests. They did not usually forage once they had helpers, although some had briefly foraged alone before additional B1 females began foraging. As well as the social nests, 8 further nests at Sussex were each reused by a single B1 daughter to produce her own offspring, the foundress having died before B1 immatures reached adulthood. These nests were solitary, but nest reuse is again a phenomenon never observed at the Belfast source site. In summary, of the 28 nests initiated at a putative social site (Sussex) by foundresses from a solitary site (Belfast), 13 became social, 8 were reused by lone B1 females, and only 7 had no first brood provisioners at all.

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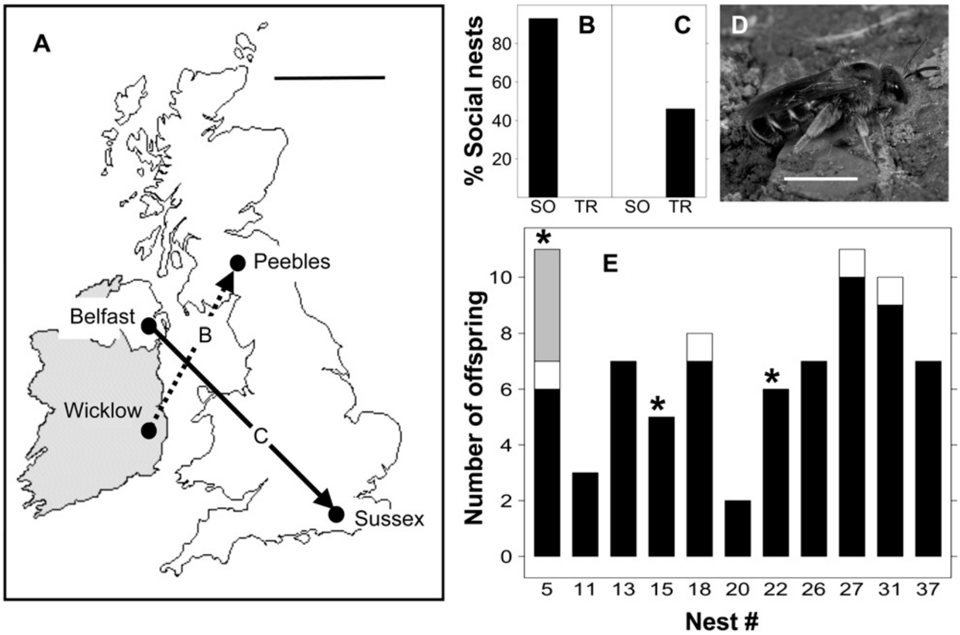

Figure 1. Social Plasticity in *H. rubicundus*

(A–C) Britain and Ireland map with arrows indicating transplants (A). Scale bar represents 150 km. Letters B and C within arrows refer to histograms showing percentage of nests initiated by foundresses from the social population at Wicklow (n = 79 nests) (B) and the solitary population at Belfast (n = 28 nests) (C) that became social at source (SO) and transplant (TR) sites.

(D) *H. rubicundus* foundress. Scale bar represents 5 mm. (Photograph © A. Gogala.)

(E) Partitioning of B2 reproduction in 11 of the social nests initiated by foundresses from the solitary population at Belfast after they had been transplanted to the putative social site at Sussex. Each bar represents a different nest. Each block within a bar represents offspring of a different mother. Asterisks indicate nests where the foundress was still alive and produced all or some (nest 5, black shading) of the B2 offspring ("eusocial"). At other nests, the foundress was dead so that B1 females were the sole mothers ("semisocial"). See also Table S1.

Social phenotype appeared to respond adaptively to at least two different aspects of the environment at Sussex, suggesting that helping was not misplaced parental care [7]. First, halictine sociality is thought to occur only where the growing season is long enough to permit the two annual broods required [4, 8, 10]. Our data further suggest that individual bees adjust their strategies according to the time remaining for offspring production: nests that produced their first female offspring earlier were more likely to have B1 provisioners (Figure 2) ($p = 0.005$). Second, nests where the foundress was still alive when B1 offspring reached adulthood had more B1 provisioners than other nests ($p < 0.015$) after controlling for the date of first offspring emergence ($p < 0.0001$) or the foundress's spring provisioning effort ($p < 0.0001$). This pattern is expected on genetic grounds: B1 females are more closely related to B2 offspring of the foundress (their mother) than to B2 offspring of other B1 females (their sisters) that replace the foundress as queen. In addition, the foundress may increase the payoff to B1 helpers by boosting group size and hence reducing the chance that the whole group will fail [15, 16].

Our findings suggest how transitions in both directions between sociality and solitary nesting could recur through expression of cryptic alternative phenotypes. Once evolved, hidden phenotypes might be expressed immediately when conditions change, as in our British and Irish *H. rubicundus*, or following selection on the environmental thresholds controlling key regulatory loci [2, 6]. The repeated accumulation of the same sets of mutations would not be required. Origins of halictine sociality are temporally linked with past episodes of warming, and sociality is today associated with warmer conditions [3, 4]. Rapid switching between social phenotypes could thus help some sweat bees to accommodate the global warming that is currently predicted, and has probably allowed them

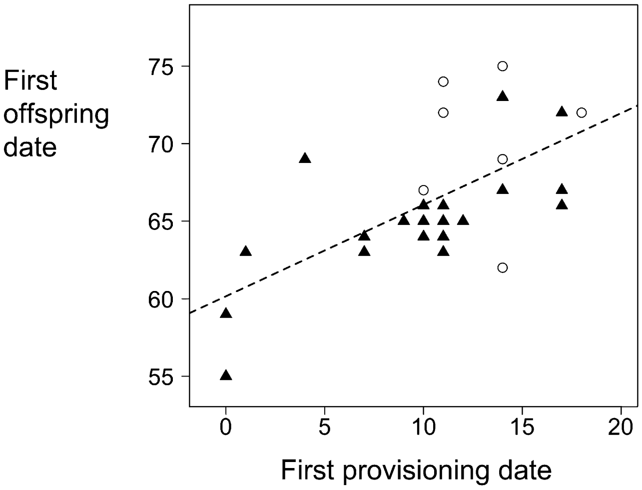

Figure 2. Relationship between First Spring Provisioning Date and Date When the First Female Offspring Reached Adulthood

Data are for nests of foundresses transplanted to the putative social site at Sussex from the solitary site at Belfast. Open circles are nests with no first brood provisioners; filled triangles are nests with first brood provisioners. Dates are days after April 22. Dashed line shows least-squares regression.

to invade new biogeographic regions [2, 10]. Interestingly, some North American *H. rubicundus* could represent a case where plasticity has been lost [4]. There is significant mitochondrial differentiation between North American populations expressing the two social phenotypes [4], whereas there is no such differentiation among British and Irish populations [13]. Loss of plasticity [2, 17–19] could involve drift or local adaptation when conditions triggering one of the phenotypes no longer occur, as at *H. rubicundus* nesting sites in the Rocky Mountains where the growing season is reliably too short to permit two annual broods [10]. Nevertheless, climatically transitional zones similar to Britain and Ireland presumably exist in North America, so that vicariance may also explain differentiation among North American phenotypes [4, 10]. For example, during past episodes of cooling, social *H. rubicundus* may have been unable to move south in parts of Europe without crossing east-west-aligned mountain ranges such as the Pyrenees. Plasticity may therefore have been favored in these populations. Where mountain ranges such as the American Rockies are aligned North-South, however, bees might have been free to move south so that plasticity was unnecessary.

In North America, sociality also appears to be more specialized than we found at Sussex. Most foundresses remain alive during provisioning of B2, and there can be two successive broods of offspring provisioners [12, 20]. B1 provisioners are also physically smaller than foundresses [8], suggesting that social phenotype is influenced earlier in development than at Sussex: B1 body size will primarily be determined by nutrition provided by the foundress at the time she lays each egg [21]. Our results show that these features are not necessary for a reproductive division of labor, but they are generally characteristic of socially more complex taxa [22, 23]. Such specialization is expected once sociality is the only phenotype exposed to selection, when features that could be detrimental if expressed alongside solitary phenotypes can more easily evolve [2]. For example, the production of small B1 females might be adaptive in an obligate social population, but could be maladaptive in a plastic population: in years when solitary nesting was expressed, such small females would become the next year's new foundresses.

There is currently considerable interest in genetic and environmental influences on sociality, and studies of obligate social taxa have revealed cooption of genes to serve new functions during social evolution [24]. *H. rubicundus* and other socially plastic taxa should make excellent models for understanding genomic changes involved in the origin of sociality itself. It is therefore worth considering what mechanisms would be required to account for the social phenotypes we observed in British and Irish *H. rubicundus*. At simplest, there might be an environmentally controlled switch that regulates diapause by responding to cues correlated with how much time remains in the growing season. If there is insufficient time remaining, a newly-emerged B1 female enters diapause (all bees in solitary populations). If there is sufficient time, however, the B1 female initiates nesting. She may then reuse her natal nest to produce her own offspring—nesting immediately without diapause is not synonymous with eusociality—many solitary bees, including some halictines [25], are bivoltine. Alternatively, however, she may become a helper (worker). Cues correlated with the prior presence of another egg-laying female must then lead to egg-laying being switched off, so that the bee becomes a specialized forager. In the foundress's absence, the above mechanisms would lead the first-emerging B1 female to begin offspring production alone. Once a second B1 female chose to begin foraging, a further mechanism would be required to suppress foraging in the first female, so that she became a specialized egg-layer. Further research may indicate that sociality in British and Irish *H. rubicundus* involves additional traits absent from solitary populations, such as the coordination of activities so that nests are not left unguarded, or signaling of reproductive status [26], as well as the maternal effects that some of our results may imply.

Experimental Procedures

Field sites comprised areas of bare soil in County Wicklow (N 52°58′, W 6°15′), Peebles (N 55°38′, W 3°10′), Belfast (N 54°32′, W 5°58′), and Sussex (N 50°52′, W 0°0′). Transplants involved collecting foundresses at source sites, just before or after winter hibernation, marking them, then placing them at the transplant site on the next sunny day, before provisioning had started in spring. Foundresses normally mate once [27], soon after maturation in autumn, then store their mate's sperm for use throughout life. Dissection showed that 23 of 25 foundresses chosen at random from autumn transplant samples were inseminated. Thus, B1 offspring produced at the transplant site will usually have carried genes entirely from the source site. Because all males die before winter, this must also have been true for spring-transplanted foundresses. Once nesting began at transplant sites, foundresses were individually marked, then all nests observed continuously on almost all days suitable for bee activity until August 1, 2006 (the solitary site at Peebles) or July 17, 2009 (the putative social site at Sussex), recording presence and entries with pollen. B1 offspring females were also individually marked and observed.

Genotyping

On July 16–17, 2009 at the putative social site in Sussex, surviving B1 provisioners were collected, then nests dissected to recover B2 immatures plus additional B1 provisioners and foundresses. Adult bees (including 60% of the B1 provisioners previously marked) and immatures were genotyped at 11 microsatellite loci developed specifically for *H. rubicundus* [28] (see Table S1 available online). Immatures were unambiguously assigned to potential mothers (adult females) using standard procedures [29]. As well as the social nests, three of the nests that were reused by a single B1 provisioner were genotyped, confirming that all B2 offspring matched the provisioner's genotype (n = 2, 9, and 10 offspring per nest).

Data Analysis

Using KINGROUP [30] software, we confirmed that all B2 offspring assigned to foundresses were significantly ($p < 0.05$) more likely to be sisters than daughters of the B1 provisioners at their nests. To compare observed reproductive skew with that expected if B2 offspring are assigned randomly among the adult females at each nest, we calculated the recommended B index using Skew Calculator 2003 software [14]. The observed value (0.47) is close to 0.57, the value obtained if all of the offspring genotyped at each nest are allocated to just one of the adult females at that nest. The significance level remains the same if the analysis is repeated taking into account the observed tenure (defined by first and last sightings) of each female [14]. All other analyses were conducted in the R package (http://www.stats.bris.ac.uk/R/). Unless otherwise stated, we used generalized linear modeling assuming Poisson, binomial, or normal errors as appropriate [31]. In each analysis we first fitted potential explanatory variables and their pairwise interactions. Starting with the interactions, we then subtracted terms from the model until further removals led to significant ($p < 0.05$) increases in deviance [31]. We report significance levels for terms when adding them last to this minimal adequate model. Foundress wing length, and whether the foundress had hibernated at her source site before transplantation, were tested as covariates in all analyses, but were never retained in the minimal model.

Supplemental Information

Supplemental Information includes one table and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2010.10.020.

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