

Mating Preference in the Commercially Imported Bumblebee species *Bombus terrestris* in Britain (Hymenoptera: Apidae)

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INGS, T C, RAINE N E & CHITTKA L [Biol Chem Sci, Queen Mary, London E1 4NS]: **Mating Preference in the Commercially Imported Bumblebees (*Bombus terrestris*) in Britain (Hymenoptera: Apidae)**. – Entomol Gener 28 (3): 233–238; Stuttgart 2005-12. – – – [Article]

Commercial trade of bumblebees in Europe results in different subspecies of *Bombus terrestris* Linnaeus 1758 being shipped into regions where they are not native. Although previous studies have shown that these subspecies will interbreed, none have assessed mating preference of the different populations. This study examines the mating preferences between two geographically isolated populations of *B terrestris* which have unnaturally been brought together through the commercial trade in bumblebees. Under controlled choice conditions, mating between commercially imported *B t dalmatinus* (from South Eastern Europe) and native British *B t audax* was non-random. Commercially imported gynes (unfertilised queens) preferred to mate with males from the same population (71% of matings). In light of the continued escape of imported gynes and males, these results indicate that there is a possibility of establishment of South Eastern European *B t dalmatinus* in Britain, and that hybrids will also occur. The ecological risks of such an establishment are discussed.

Key words: *Bombus terrestris* Linnaeus 1758 – *B t audax* (Harris 1776) – *B t dalmatinus* (Dalla Torre 1882) – hybridisation – invasion – mate choice

INGS T C, RAINE N E & CHITTKA L [Biol Chem Sci, Queen Mary, London E1 4NS]: **Partnerwahl-Präferenzen bei der kommerziell importierten Hummel-Art *Bombus terrestris* in Großbritannien (Hymenoptera: Apidae)**. – Entomol Gener 28 (3): 233–238; Stuttgart 2005-12. – – – [Abhandlung]

Der kommerzielle Handel mit Hummeln in Europa hat zur Folge, daß Unterarten von *Bombus terrestris* Linnaeus 1758 in Regionen verbracht werden, in denen sie nicht heimisch sind. Frühere Untersuchungen haben gezeigt, daß diese Unterarten kreuzbar sind, aber keine Studie hat bisher die Präferenzen bei der Partnerwahl bei Hummeln quantitativ untersucht. In der hier vorliegenden Arbeit werden die Präferenzen bezüglich der Partnerwahl bei zwei Hummelunterarten geprüft, die in der Natur geographisch isoliert sind, aber durch kommerzielle Verschiffung in Kontakt gekommen sind. Unter kontrollierten Wahlbedingungen waren die Paarungen zwischen kommerziell importierten *B t dalmatinus* (aus Südosteuropa) und einheimischen britischen *B t audax* nicht zufällig verteilt. Königinnen der importierten Population zogen ♂♂ der gleichen Population vor (71% der Kopulationen). In Anbetracht der Tatsache, daß Königinnen und ♂♂ der importierten Hummelvölker oft aus Gewächshäusern entweichen, ergibt sich aus diesen Beobachtungen die Möglichkeit, daß sich südosteuropäische *B t dalmatinus* in Großbritannien ansiedeln, und daß außerdem Hybride auftreten. Die ökologischen Risiken einer solchen Einbürgerung werden diskutiert.

Schlüsselbegriffe: *Bombus terrestris* Linnaeus 1758 – *B t audax* (Harris 1776) – *B t dalmatinus* (Dalla Torre 1882) – Hummel – Hybridisierung – Invasion – Partnerwahl

1 Introduction

Bumblebees are economically important pollinators of glasshouse crops such as tomatoes and sweet peppers [ERCAN & ONUS 2003, CARRUTHERS 2004]. The high demand for bumblebees from crop growers has led several companies to commercially rear colonies of the bumblebee species *Bombus terrestris* Linnaeus 1758. As a consequence, since the early 1990's [VELTHUIS 2002], many thousands of these commercial colonies are being shipped throughout Europe every year [INGS, SCHIKORA & CHITTKA 2005]. Although *B terrestris* is a common and widespread species in Europe, nine separate subspecies are recognised to occur in different regions [AYTEKIN, RASMONT & ÇAĞATAY 2003]. In fact, these subspecies show significant variation in a number of important traits, including coat colour, floral colour preference and foraging performance [CHITTKA & BRISCOE 2001, CHITTKA, INGS & RAINE 2004, INGS et al 2005]. Unfortunately, the current commercial trade in *B terrestris* involves the regular shipment of subspecies into regions where they are non-native [ESTOUP, SOLIGNAC, CORNUET et al 1996, INGS et al 2005].

This is of concern because although commercial bumblebee colonies are generally used in glasshouses, bees, including sexually reproductive males and queens, are escaping into the wild [MORANDIN, LAVERTY, KEVAN et al 2001, GOULSON, HUGHES, DERWENT et al 2002]. As a consequence, there is a risk that non-native populations could become established and have negative impacts on native populations, e.g through competition for food [THOMSON 2004, INGS et al 2005]. However, there is also a risk that hybridisation between introduced and native populations may occur, ultimately leading to a loss of genetic diversity of European *B terrestris* [ESTOUP et al 1996, WIDMER, SCHMID-HEMPEL, ESTOUP et al 1998]. In fact, evidence from DE JONGHE [1986] and the data presented here (**Tab 1**) shows that populations will interbreed and produce viable colonies in the next generation. However, to date, no studies have actually quantified mate choice between populations in a controlled manner.

Therefore, the aim of this study is to determine the mating preferences of two geographically isolated populations of *B terrestris* which have unnaturally been brought together because of the commercial trade in bumblebees.

2 Material and methods

2.1 Study populations

Currently, the South Eastern European (e.g Turkey and Greece) subspecies *B terrestris dalmatinus* (Dalla Torre 1882) is being imported into the United Kingdom where *B t audax* (Harris 1776) is the native subspecies. Therefore, young commercially produced colonies of *B t dalmatinus* were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) and native *B t audax* colonies were reared from nest-searching queens captured in the Greater London area in southern Britain during March 2005.

Unfertilised queens (gynes) and males from both commercial and native populations were collected from colonies that were allowed to mature in the field during the summer of 2005. After removal from their parent colonies, all gynes and males were kept in separate wooden boxes in a dark room and were fed with pollen and sugar syrup (obtained from Koppert Biological Systems) *ad libitum*.

Tab 1: Hybridisation success between populations of the bumblebee species *Bombus terrestris* (Linnaeus 1758). The number of gynes from each potential hybrid mating which were successfully mated, and subsequently overwintered under laboratory conditions are indicated where possible. Instances in which overwintered, mated queens established viable colonies (i.e containing workers) is also presented. Those hybrid matings for which no viable colony production are presented have not established successful colonies in our laboratory to date. Data presented pertaining to matings between *B t terrestris* and *B t xanthopus* (including those involving the F1 progeny of such matings) are from DE JONGHE [1986], and are indicate by †. All other data were collected by one (or more) of the authors.

B. terrestris subspecies			Number of queens:		Viable colony
Gynes		Males	a) mated	b) overwintered	produced?
<i>audax</i>	x	<i>dalmatinus</i>	3	3	
<i>canariensis</i>	x	<i>dalmatinus</i>	2	0	
<i>dalmatinus</i>	x	<i>audax</i>	10	10	yes
<i>dalmatinus</i>	x	<i>canariensis</i>	26	26	yes
<i>dalmatinus</i>	x	<i>sassaricus</i>	18	16	yes
<i>dalmatinus</i>	x	<i>terrestris</i>	-	>10	yes
<i>sassaricus</i>	x	<i>terrestris</i>	-	2	yes
<i>terrestris</i>	x	<i>dalmatinus</i>	-	>10	yes
<i>terrestris</i>	x	<i>sassaricus</i>	-	4	yes
<i>terrestris</i>	x	<i>xanthopus</i>	-	4	yes †
<i>xanthopus</i>	x	<i>terrestris</i>	-	8	yes †
<i>xanth. x terr. F1</i>	x	<i>terrestris</i>	-	2	yes †
<i>xanth. x terr. F1</i>	x	<i>xanth. x terr. F1</i>	-	2	yes †

2.2 Female mate choice

Four gynes, of a particular population, aged between 3 and 20 days old, were randomly selected and placed in a mating chamber in a dark room. Mating chambers used were clear plastic fish tanks (Marchioro, Vicenza, Italy: 180 x 200 x 285 mm) lined with a thin layer of "Fullers Earth" cat litter on the floor. Both nectar and pollen were constantly available to males and gynes in all mating chambers.

Males, aged between 5 and 20 days old, were selected at random from colonies unrelated to the gynes for mate choice experiments. Each male was marked on the thorax with a uniquely numbered, white tag (Opalith Plättchen, Christian Graze KG, Weinstadt-Endersbach, Germany) to enable individuals to be identified. In each mating chamber, four gynes were presented with a choice of four males from each subspecies (*B t dalmatinus* and *B t audax*), eight males in total, producing an overall gyne to male ratio of 1:2. Once males were added, the mating chambers were placed in natural daylight, and were regularly monitored (constantly for the first hour (09:00–10:00 hrs), and at least every ten minutes thereafter until 15:00 hrs). As soon as a copulation event was observed, the copulating pair were removed from the mating chamber, and replaced with a fresh gyne and male from the correct subspecies. If either a gyne or a male did not copulate within three days it was replaced with another from the correct subspecies.

Unfortunately due to the low incidence of gyne production by *B t audax* colonies there were insufficient gynes to carry out male mate choice experiments.

Tab 2: Summary of successful matings of gynes from commercial *B t dalmatinus* and native *B t audax* given an equal choice between males of both subspecies.

	subspecies	Males	
		audax	dalmatinus
Gynes	<i>audax</i>	–	1
	<i>dalmatinus</i>	14	34

3 Results

As soon as males were added to the mating chambers, males of both subspecies quickly attempted to mate with both *B t dalmatinus* and *B t audax* gynes by climbing on their backs and extending their genital capsule. Males varied in how persistently they attempted to mate with gynes, although there were no obvious differences in motivation between subspecies. The vast majority of all mating attempts were unsuccessful, with the gynes actively trying to remove males from their backs using their legs, or by running away. Copulation only occurred when gynes extended their sting to allow males to insert their genital capsule.

Forty-nine gynes (48 *B t dalmatinus* and 1 *B t audax*) mated successfully (**Tab 2**) out of 71 tested (68 *B t dalmatinus* and 3 *B t audax*). Both combinations of cross matings occurred, i.e. *B t dalmatinus* gynes with *B t audax* males, and *B t audax* gynes with *B t dalmatinus* males. However, *B t dalmatinus* gynes preferentially mated with males from the same subspecies as themselves (71%, $\chi^2 = 8.33$, $p < 0.005$). Unfortunately only one *B t audax* gyne successfully mated (with a *B t dalmatinus* male) so no inferences about mate choice could be made for this population.

4 Discussion

Previous studies have shown that although subspecies of *B terrestris* have diverged in a number of traits [CHITTKA & BRISCOE 2001, CHITTKA et al 2004, CHITTKA & WELLS 2004, INGS et al 2005] they will interbreed and produce viable offspring under artificial conditions [DE JONGHE 1986; **Tab 1**]. However, in these instances gynes were not given a choice between males from more than one subspecies. Therefore, it is important to ask what might occur under such choice conditions. Evidence from the island of Elba [RASMONT & QUARANTA 1997], where two subspecies coexist, and from personal observations in southern France, where several subspecies coexist, suggests that hybridisation between subspecies does occur under natural conditions. Yet this still does not indicate what choices gynes are making when mating.

This study provides the first strong evidence that mating in a geographically isolated pair of subspecies (*B t dalmatinus* from South Eastern Europe and *B t audax* from the UK) is non-random, when the two subspecies are brought together under controlled conditions. Commercially imported *B t dalmatinus* gynes showed a strong mating preference for males from their own subspecies, although they clearly can and will mate with native British (*B t audax*) males. One plausible explanation for these observations is that assortative (directional) mating is present, as expected between populations that have undergone a period of independent evolution [GANEM, GINANE, OSTROWSKI et al 2005].

An alternative explanation why *B t dalmatinus* gynes preferentially select males from the same subspecies as mates, is that *B t dalmatinus* males are more “attractive” to gynes of both subspecies than *B t audax* males. For instance, gynes could be responding to subspecific differences in their pheromone profiles. To ascertain which of these is actually occurring, it is necessary to determine what, if any, is the mating preference of *B t audax* gynes. Unfortunately, this was not possible in this study as only one of three available *B t audax* gynes mated, albeit with a *B t dalmatinus* male.

Although the mechanism for the preferential mate choice cannot be elucidated from these results, they clearly have important implications for the potential invasiveness of commercial bumblebees. Firstly, as commercial *B t almatinus* gynes prefer to mate with males of their own subspecies this could increase the chances of pure *B t dalmatinus* becoming established in the wild in the UK. Furthermore, as pure *B t dalmatinus* colonies perform considerably better than native British *B t audax*, both in terms of their nectar foraging performance and colony reproductive success (INGS et al in prep), there is a real risk that they could displace native *B t audax*. Secondly, the fact that about one third of the *B t dalmatinus* gynes mated with *B t audax* males suggests that there is a high risk of hybridisation between commercial and native bees. Such hybridisation could lead to introgression of maladaptive genes, e.g low levels of parasite resistance, from the commercially reared bees – potentially leading to population crashes of native populations, a phenomenon which has recently been observed in salmon [HEATH, HEATH, BRYDEN et al 2003, MCGINNITY, PRODOHL, FERGUSON et al 2003]. Alternatively hybrids may have increased vigour compared to their parents [LEVIN, FRANCISCO-ORTEGA & JANSEN 1996, PERRY, LODGE & FEDER 2002, FACON, JARNE, POINTIER et al 2005] and have negative impacts on native bumblebee populations through resource competition [THOMSON 2004].

In conclusion, this study has shown that the mate choice between two geographically isolated populations of *B terrestris* subspecies is not random, but commercially imported bees (*B t dalmatinus*) can, and will, mate with native (*B t audax*) bees. This has important implications for the current importation of non-native populations of *B terrestris* for pollination of glasshouse crops. Not only will imported bees hybridise with the native population, but there is also an increased risk that pure non-native populations will become established. Although these results focus on the UK, they clearly have much wider implications throughout Europe where the mating preference of different populations might vary considerably. Further work on mate preference between the nine European populations of *B terrestris* is not only important in terms of assessing the invasive risks associated with the commercial importation of *B terrestris*, but will also aid our understanding of the importance of mate choice in evolutionary processes.

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