Special Feature for Ecological Risk Assessment of Introduced Bumblebees

Reproductive disturbance risks to indigenous Japanese bumblebees from introduced *Bombus terrestris*

Koji Tsuchida,1,* Natsuko Ito Kondo,2 Maki N. Inoue2 and Koichi Goka2

1 Laboratory of Insect Ecology, Faculty of Applied Biological Sciences, Gifu University; Gifu, Gifu 501–1193, Japan
2 National Institute for Environmental Studies; Tsukuba, Ibaraki 305–0053, Japan

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Abstract
The bumblebee *Bombus terrestris* is not only an effective pollinator but also a potential invasive species outside its native range. Interspecific crossing of *B. terrestris* with indigenous *B. hypocrita sapporoensis* and *B. ignitus* can cause inviable hybrid production under laboratory conditions. Additionally, nearly 30% of *B. hypocrita sapporoensis* and *B. hypocrita hypocrita* queens are estimated to copulate with *B. terrestris* males in the field, suggesting that genetic deterioration of indigenous bumblebees is possible through hybridization with the introduced species. We briefly review interspecific reproductive isolation mechanisms and discuss the negative impacts of *B. terrestris* on the reproduction of indigenous bumblebee species in Japan due to interspecific mating.

Key words: *Bombus terrestris*; invasive species; hybridization; reproductive disturbance

INTRODUCTION

Bumblebees are important pollinators of wild flora as well as agricultural crops. *Bombus terrestris*, which originated in Europe, has been successfully mass reared following the methodology of Röseler (1985) and van den Eijnde et al. (1991), and is used as an effective commercial pollinator in greenhouses all over the world. In 1991, *B. terrestris* was deliberately introduced into Honshu, Japan, for experimental purposes, and in 2004, about 70,000 colonies were released into greenhouses throughout Japan (Kunitake and Goka, 2006). Pollination by *B. terrestris* reduces the need for laborious hand pollination and chemical hormones and, concomitantly, insecticides (Velthuis and van Doorn, 2006).

The unrestricted mass release of *B. terrestris* into novel environments, such as Japan, influences native fauna and flora when *B. terrestris* escapes from greenhouses. Monitoring of wild *B. terrestris* nests in Japan indicates that *B. terrestris* has become naturalized in Japan (Matsumura et al., 2004; Nakajima et al., 2004). Four negative ecological impacts on the native fauna and flora are predicted: (1) interspecific competition between *B. terrestris* and native bumblebee species for food resources and nesting sites (Inari et al., 2005; Makino and Sakai, 2005; Ings et al., 2006; Inoue et al., 2008), (2) the possibility that parasites of *B. terrestris* might also escape and might affect native bumblebee species (Goka et al., 2001, 2006), (3) disruption of mutualisms between native plants and native pollinators (Dohzono et al., 2008), and (4) genetic deterioration due to interspecific mating between *B. terrestris* and native species (e.g., Rhymer and Simberloff, 1996).

In Japan, 21 native bumblebee taxa are known (Washitani et al., 1997). In 2006, the *Invasive Alien Species* Act (Law No. 78, Ministry of the Environment, Government of Japan) was first enforced, and *B. terrestris* has been designated as an invasive alien species in Japan based on both field and laboratory studies. Multiple studies have documented the unequivocal negative ecological impacts of *B. terrestris*, including inviable hybrid production re-
sulting from matings between introduced \textit{B. terrestris} and two indigenous Japanese species, \textit{B. hypocrita sapporoensis} and \textit{B. ignitus}, in the laboratory. In addition, 20.2 and 30.2% of wild-caught queens of \textit{B. h. hypocrita} (allopatrically distributed with \textit{B. h. sapporoensis} in Honshu, Japan) and \textit{B. h. sapporoensis}, respectively, had stored spermatozoa from \textit{B. terrestris} males, indicating that approximately 30% of indigenous bumblebees copulate with \textit{B. terrestris} in the field (Kondo et al., 2009). Here, we briefly review hybrid production in light of the reproductive isolation mechanisms that have been observed in other animals, and evaluate the influences of inviable hybrid production on the reproduction of bumblebees indigenous to Japan.

**HYBRIDIZATION AND REPRODUCTIVE ISOLATION**

Some authors focus on hybridization as a potential source of genetic variation, functional novelty, and new species. Hybrid speciation is more common in plants and animals than previously thought (e.g., Mallet, 2005, 2007; Mavárez and Linares, 2008): at least 10% of animal species are involved in hybridization and potential introgression with other species. On the other hand, hybridization between native and alien species potentially generates complete loss of the genome complex and extinction of the indigenous species (Rhymer and Simberloff, 1996).

Interspecific reproductive isolation mechanisms can be categorized as pre-mating, post-mating/pre-zygotic, or post-zygotic isolation mechanisms. If these isolating mechanisms are incomplete, hybridization might take place. Hybrid inviability results in the death of hybrid individuals at some point during development. Hybrid sterility is the inability of a hybrid individual to produce viable offspring, even when the hybrid individual survives to adulthood.

**PRE-MATING ISOLATION**

The first step in mating is the search for mating partners, in which visual, auditory and olfactory signals play important roles. In some species of African cichlid fishes, visual cues play important roles in mate identification (Knight and Turner, 2004). If water turbidity increases and results in decreased visibility, this reproductive isolation mechanism does not work as effectively (See hausen et al., 1997, 2008). In Hawaiian \textit{Laupala} crickets, auditory signals seem to have been key factors in sympatric speciation; rapid species expansion has been driven by sexual selection through calling for mates (Mendelson and Shaw, 2005). In many insect species, sex pheromones are important in mate identification (Smadja and Butlin, 2009). In two closely related \textit{Drosophila} species, sexual selection is largely based upon differences in female cuticular hydrocarbons (i.e., contact pheromones). This difference maps to only one of the three major chromosomes, implying that reproductive isolation might have a fairly simple genetic basis (Coyne et al., 1994). In several related \textit{Ostrinia} and \textit{Yponomeuta} moth species, the ratio of pheromone constituents and/or female calling times varies interspecifically (Roelofs et al., 1987; Löfstedt et al., 1991; Ishikawa et al., 1999). Löfstedt (1993) argued that evolutionary changes in moth pheromone and receptor systems began with the production of a new female signal, followed by changes in male preference.

In bumblebees, preliminary observations of interspecific matings of \textit{B. ignitus} gynes \times \textit{B. terrestris} males revealed that 70.3% of \textit{B. ignitus} gynes (26/37) successfully copulated under laboratory conditions (Kanbe et al., unpublished data). This is similar to the percentage of intraspecific matings of \textit{B. terrestris} under similar laboratory conditions (69–80%; Duchateau, 1985; Tasei et al., 1998). The percentage of successful copulations of \textit{B. hypocrita sapporoensis} gynes \times \textit{B. terrestris} males should be nearly 50%, based on the ease of inducing copulation (Kanbe et al., 2008).

These high percentages of interspecific matings could be explained by both the lability of species discrimination at close range and the partial overlap of male sex pheromones. In bumblebees, both male and female sex pheromones are important for copulation. In \textit{B. terrestris}, female- and male-produced compounds act as sex pheromones. Males establish flight routes that they patrol and along which they deposit species-specific scent marks that act as pre-mating attraction signals to virgin queens (Ayasse et al., 2004). After a virgin queen has landed in the patrol area of a male, the female pheromone stimulates the mating behavior of males in close vicinity. Krieger et al. (2006) identi-
fied the female sex pheromone of *B. terrestris* and stressed that female sex pheromones are responsible for eliciting copulation behavior from conspecific males at close range. Ono and Wada (1996) reported interspecific activity in the female sex pheromones of *B. ignitus* and *B. h. hypocrita*, whereas no interspecific activity of male sex pheromones occurs in these two sympatric species. This finding suggests that (1) bumblebees discriminate conspecific mating partners via male sex pheromones, and (2) the species discrimination ability of virgin gynes and males is disrupted at close range.

Kondo et al. (2009) reported that 20.2 and 30.2% of *B. h. hypocrita* and *B. h. sapporoensis* queens, respectively, stored spermatozoa from *B. terrestris* males. This finding might be explained by the similarities of male sex pheromones; although the constituents of male sex pheromones of native Japanese species differ, those of *B. terrestris* might partially overlap with those of *B. h. hypocrita*, *B. h. sapporoensis*, and *B. ignitus* (Ono, personal communication). *Bombus terrestris*, *B. ignitus*, and *B. hypocrita* are phylogenetically closely related (Kawakita et al., 2004; Cameron et al., 2007). Coyne and Orr (1989) suggest that pre-zygotic reproductive isolation may be reinforced when allopatric taxa become sympatric. Cephalic labial gland secretions can be recognized by females as species-specific cues in the sympatric species pair *B. promorum* and *B. mesomelas* (Terzo et al., 2007). These lines of evidence suggest that, due to their previous allopatric distribution, *B. terrestris* males (or *B. h. hypocrita* and *B. ignitus* males) have not yet evolved mechanisms to reproductively isolate them from allopatric species not previously encountered. Another possibility is that *B. terrestris* males evolved pheromonal variation through artificial selection across many generations in greenhouses; they might have lost key constituents of the pheromone blend through artificial “indeliberate” selection.

**POST-MATING/PRE-ZYGOTIC ISOLATION**

Upon copulation, intersexual and intrasexual competition could drive the evolution of some special characters in both sexes. In some insects, most of the sperm deposited by the first male to mate with a female are displaced by the sperm of males that subsequently mate with that female (i.e., sperm displacement; Waage, 1979). Apyrene sperm or seminal fluid in mated females can delay remating. In the army worm *Pseudaletia separata*, males transmit two types of sperm to females, eupryne and apyrene; the latter prevent a female from re-mating (He et al., 1995). Similar results were obtained in the white butterfly *Pieris napi* (Cook and Wedell, 1999). In *Drosophila*, mated females are less attractive to males and have decreased receptivity to further matings due to a sex peptide transmitted by males (Wollner, 1997). *Drosophila melanogaster* can both physically displace and incapacitate stored sperm from males with which a female has previously mated (Price et al., 1999). However, a more recent study by Snook and Hosken (2004) revealed that seminal fluid does not kill rival sperm in *Drosophila*. In species other than lepidopterans and dipterans, seminal substances can disrupt successful mating: in the seed bug *Togo hemipterus*, accessory gland substances from males inhibit the mating receptivity of virgin females (Himuro and Fujisaki, 2008); in the ground beetle *Leptocarabus procerulus*, seminal substances both act as a mating plug and induce female refractory behavior (Takami et al., 2008). These substances could evolve via sexual selection for mating disruption, and such competition could generate reproductive barriers between closely related species (Martin-Coello et al., 2009).

In bumblebees, males transfer sperm with a sticky gelatinous product from their accessory gland upon copulation. The sticky product, whose main constituent is a linoieic acid (Baer et al., 2000, 2001), not only prevents the backflow of sperm, but also decreases sperm transfer into the female’s spermatheca and prevents second matings in *B. terrestris* (Duvoisin et al., 1999; Sauter et al., 2001). This result explains why *B. terrestris* queens are monandrous, although multiple mating is beneficial at times during the colony cycle with respect to parasitism and fitness (Baer and Schmid-Hempel, 1999). Alternatively, polyandry also induces a cost to a colony (Baer and Schmid-Hempel, 2001). Either way, most bumblebee species are strictly monandrous (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000; Payne et al., 2003) with the exception of *B. hypnorum*, which is polyandrous (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000;
Brown et al., 2002). Furthermore, B. hypnorum males have longer sperm than monandrous bumblebees, which might reflect sperm competition among males (Baer et al., 2003). Another possible explanation of polyandry in B. hypnorum is that the mating plug does not last as long as in B. terrestris (Brown et al., 2002).

Not much is known about the interspecific activity of mating plugs. During interspecific mating in bumblebees, we confirmed the existence of sperm in the spermathecae of mated females (Kanbe et al., 2008). Therefore, a mating plug is transmitted to females in interspecific matings. Although we do not have any genetic data regarding the mating frequencies of B. h. sapporoensis and B. ignitus queens, several of the studies mentioned above suggest that monandry is predominant. If the mating plug has interspecific activity, interspecific mating with B. terrestris could prevent further interspecific mating by queens of the second species. Further studies are needed to evaluate the interspecific activity of the primary mating plug substance, linoleic acid.

**POST-ZYGOTIC ISOLATION**

Hybridization between different species can result in hybrid inviability or hybrid sterility, and the probability of a successful interspecific cross decreases with divergence between the hybridizing species (Coyne and Orr, 1989, 1997, 1998; Presgraves, 2002; Price and Bouvier, 2002; Lijtmaer et al., 2003; Bolnick and Near, 2005). Positive selection for reproductive isolation genes (e.g., the candidate gene *Odysseus*) is responsible for the evolution of hybrid male sterility in Drosophila (Ting et al., 1998).

The Dobzhansky–Müller model explains how populations become reproductively isolated without passing through an unfit maladaptive stage with inviability and sterility by assuming negative epistatic interactions between pairs of genes. The model assumes that ancestral populations have two interacting loci (*aabb*), where the *AaBb* genotype is inviable or sterile due to a detrimental interaction between *A* and *B*. Then, two independent populations diverge from the original population and bear genotypes *Aabb* and *aaBb*. If alleles *A* and *B* become fixed in each population, respectively, due to positive selection or drift, then either *AAbb* or *aaBB* becomes fixed in each population. If the two populations later come into contact, the resulting hybrid genotype *AaBb* is inviable or sterile (see Hayashi and Kawata, 2002 for a detailed explanation). Recently, loci exhibiting the Dobzhansky–Müller effect were detected in Drosophila (Brideau et al., 2006) using a mutant strain of *D. simulans* that could hybridize with *D. melanogaster* and produce viable hybrids (see Sawamura and Tomaru, 2002). In haplo-diploid organisms, the Dobzhansky–Müller effect could also explain partial incompatibility between two populations of the spider mite *Tetranychus quercivorus*, in which the incompatibility could be caused by an interaction between the cytoplasm from the Sapporo population and a single nuclear gene from the Tsukuba population. Compatibility is not restored when individuals from the Sapporo population are treated with antibiotics or high temperature, indicating that the cytoplasmic factors are possibly cytoplasmically inherited elements such as mitochondria rather than microorganisms (Gotoh et al., 1995). Similarly, negative epistatic interactions between nuclear and mitochondrial genes could be responsible for hybrid breakdown in parasitoid *Nasonia* wasps (Ellison et al., 2008; Niehuis et al., 2008).

Another frequently observed pattern of post-zygotic isolation reflects Haldane’s rule (Coyne and Orr, 1989, 1997, 1998; Presgraves, 2002; Price and Bouvier, 2002; Lijtmaer et al., 2003; Bolnick and Near, 2005). Haldane’s rule states that if only one gender of hybrids is inviable or sterile in an interspecific cross, it is nearly always the heterogametic sex (Laurie, 1997). Haldane’s rule can be viewed as a consequence of negative epistatic interactions between complementary genes, similar to the assumptions of the Dobzhansky–Müller effect. Suppose that one of the complementary genes is located in the *X* chromosome. Then, *AYBb* (i.e., the heterogametic sex) may be negatively affected, whereas *AaBb* is not, if the presence of *A* provides protection from the potentially harmful effects of the *A–B* interaction.

Two other mechanisms could explain Haldane’s rule: the faster male theory and the faster *X* theory. The faster male theory predicts that, because males evolve faster than females due to sexual selection, male hybrids have a higher chance of having disrupted gene interactions and thus show more incompatibilities than female hybrids. Based on a de-
lberate mechanical sex reversal experiment, leaving genetic sex unchanged, the faster male hypothesis explains sex-biased hybrid breakdown in hybrid Xenopus frogs (Malone and Michalak, 2008). The faster X theory predicts that the X chromosome has a larger effect on incompatibility than do autosomes, because many of the regions involved in hybrid incompatibility are located on the X chromosome. Koevoets and Beukeboom (2009) recently stressed that in haplo-diploid insects, haploid males (sons of F₁ hybrid females) could also suffer the negative effects of hybridization more than diploid females.

In hymenopterans, several cases of hybrid production have been observed. Seifert (1999) estimated the extent of hybridization in the ant fauna of Central Europe using high-precision stereomicroscopy and concluded that 17 of 164 ant species definitely hybridized. However, hybrid production has not been confirmed with genetic methodology in many species (or subspecies) pairs.

Hybrid production in social Hymenoptera can be divided into three categories: (1) hybrid production without introgression, (2) hybrid production with introgression, (3) social hybridogenesis.

First, hybrid production without introgression means that interspecific mating results in hybrid worker emergence, but lacks hybrid queen reproduction, and males of the maternal species are parthenogenetically produced. The ant Lasius niger hybridizes with its congener L. alienus and produces hybrid workers (Pearson, 1983); furthermore, some nests in the field are occupied by colonies with hybrid workers (Pearson, 1987). However, colony founding by hybrid queens has not been confirmed (Umphrey, 2006). Similarly, using allozyme markers, Umphrey and Danzmann (1998) found that Lasius latipes hybridize with L. claviger and found no evidence that hybrid queens found colonies. Presumably, these hybrid queens cannot produce queen-size females due to the negative effects of heterospecific males, but can produce males without the negative effects of parthenogenetic reproduction. These interspecific matings can be considered adaptive, as the “best of a bad situation”, when conspecific mating partners cannot be found (Umphrey, 2006).

Second, hybrid production with introgression means that interspecific mating results in the emergence of both hybrid workers and queens. Imported Solenopsis invicta and S. richteri from South America hybridize, and introgression occurs in North America (Ross et al., 1987). Increased fluctuating asymmetry is observed in hybrid individuals, suggesting that a barrier to gene flow between species is caused by weak selection against hybrids (Ross and Robertson, 1987). Interestingly, the two species do not hybridize within their native range (Ross and Shoemaker, 2005).

Third, social hybridogenesis means that, although worker castes are produced by hybridization between two species, queens are produced by intraspecific mating (i.e., genetic caste determination). Social hybridogenesis is similar to but not same as hybridogenesis; in the latter, the paternal heterospecific genome is expressed in somatic tissue but discarded in the germ line (Vrijenhoek, 1993). In social hybridogenesis, the paternal heterospecific genome is expressed in somatic tissue and only retained in workers. For example, when S. xyloni queens mate with S. geminata males, the resulting workers are F₁ hybrids, whereas nearly all the winged queens are of pure S. xyloni ancestry (Helms Cahan and Vinson, 2003). Thus, social hybridogenesis that results in loss of worker potential in pure-species offspring necessitates hybridization for worker production, but prevents hybrids from being represented in the reproductive caste.

A similar phenomenon is observed in populations of Pogonomyrmex harvester ants (Helms Cahan et al., 2002; Julian et al., 2002; Volny and Gordon, 2002). Social hybridogenesis evolved in these populations after complex hybridization events, and four distinct genetic lineages have been identified, each consisting of unique blends of the genomes of the parental species, presumably P. barbatus and P. rugosus. Inter-lineage hybridization between lineages H1 and H2 and between J1 and J2 can produce workers, whereas queens develop from intra-lineage matings (Helms Cahan and Keller, 2003). In social hybridogenesis, pure lineage reproducitives (queens and males) are produced; in contrast, in hybrid production without introgression, only pure lineage males are produced. Two models have been proposed to explain the genetic caste determination system in social hybridogenesis, which are similar to the Dobzhansky–Muller model (Helms Cahan and Keller, 2003; Linksvayer et al., 2006).

Mitsuhata and Ono (1996) reported that, under
laboratory conditions, *B. terrestris* could successfully produce hybrid workers with indigenous *B. h. sapporoensis*. However, Kanbe et al. (2008) found that *B. terrestris* gyne × *B. h. sapporoensis* male matings produced hybrid diploid eggs, but no diploid larvae hatched. Histological studies of eggs derived from interspecific matings revealed termination of development in the egg, resulting in inviability. This result also indicates that no danger exists of genetic introgression of the gene pool of indigenous *B. h. sapporoensis*. Similar results were obtained in crosses between *B. ignitus* and *B. terrestris*, in which hybrid diploid eggs terminated development within a few days, and only male larvae successfully hatched (Tsuchida et al., in prep.). Because *B. terrestris*, *B. ignitus*, *B. h. hypocrita*, and *B. h. sapporoensis* have the same number of chromosomes (*n*=18), imbalanced gametogenesis can be ruled out as a cause of hybrid inviability (Yamaguchi, unpublished data). The hybrid production reported by Mitsuhata and Ono (1996) might have been caused by automictic parthenogenesis with central fusion, as observed in the Cape honeybee (Greeff and Villet, 1993; Oldroyd et al., 2008), or a mutation at a locus (or loci) causing hybrid inviability. At present, because no evidence exists of hybrid production in bumblebees other than the report by Mitsuhata and Ono (1996), it is likely that hybrid worker production between introduced *B. terrestris* and indigenous *B. ignitus* and *B. h. sapporoensis* would be negligible. Therefore, most crossing between introduced *B. terrestris* and indigenous Japanese bumblebees would not lead to diploid worker production, and any resulting colonies should collapse without production of any reproductives.

As previously mentioned, Kondo et al. (2009) analyzed the DNA sequence of spermatozoa stored in queens’ spermathecae of indigenous bumblebees in Japan and found that 20.2 and 30.2% of *B. h. hypocrita* and *B. h. sapporoensis* queens, respectively, stored spermatozoa from *B. terrestris* males. This finding strongly indicates that interspecific matings between introduced *B. terrestris* and indigenous *B. h. hypocrita* and *B. h. sapporoensis* are indeed occurring in the field, and interspecific mating could lead to population extinction of indigenous bumblebees.

**CONCLUSION AND PERSPECTIVE**

Introduced *B. terrestris* and indigenous *B. ignitus*, *B. h. hypocrita*, and *B. h. sapporoensis* in Japan are phylogenetically related (Kawakita et al., 2004; Cameron et al., 2007). However, *B. terrestris* was originally allopatrically distributed with respect to the other three species. We briefly reviewed interspecific reproductive isolation mechanisms, namely, pre-mating, post-mating/pre-zygotic, and post-zygotic isolation mechanisms. Among these mechanisms, two are thought to have roles in interspecific mating and hybrid inviability in bumblebees. First, pre-mating isolation mechanisms do not prevent interspecific mating of bumblebees in the field because of bees’ historical allopatric distribution and resulting similarity in sex pheromones. Second, a post-zygotic isolation mechanism prevents normal gametogenesis and is an evident barrier to gene flow between the bumblebee species; gametogenesis is disturbed by interspecific mating, and colonies started by heterospecifically inseminated queens should collapse without producing new queens or workers. Production of inviable hybrids is functionally similar to the sterile insect technique (SIT), which uses male insects irradiated with γ rays. In Japan, the invasive melon fly *Bactricera cucubitae* was successfully eradicated using SIT (Koyama et al., 2004). Wild females that mate with irradiated males cannot produce fertilized eggs; hence, the population density declines, and the species eventually becomes eradicated. Mating disruption caused by *B. terrestris* could be viewed as another type of SIT.

The *Invasive Alien Species Act* (Law No. 78, Ministry of the Environment, Government of Japan) was enacted in Japan in 2006. Before its enforcement, no regulations were in place preventing *B. terrestris* from escaping from greenhouses. The documentation of wild *B. terrestris* nests in Japan indicates that *B. terrestris* became naturalized before 2006 (Matsumura et al., 2004; Nakajima et al., 2004). Yokoyama et al. (2004) reported a mass infestation of *B. terrestris* queens in an area with many sources of bumblebees introduced for greenhouse tomato cultivation in Hokkaido. Inoue et al. (2008) reported an increase in the *B. terrestris* population at the expense of *B. h. sapporoensis*. Similarly, several authors have documented negative ecological impacts of *B. terrestris* on native flora.
(Inari et al., 2005; Kenta et al., 2007; Dozhono et al., 2008) and a declining population of native bumblebees, whose niche overlaps with that of B. terrestris (Inoue et al., 2008; Ishii et al., 2008). A substantial number of B. terrestris males escape from greenhouses in Hokkaido, with B. terrestris males outnumbering B. h. sapporoensis males by a factor of more than 50 in the autumn mating season (Inoue et al., 2008). Both the lability of species discrimination and the numerical predominance of B. terrestris males in the mating pool could be responsible for the substantial frequency of interspecific mating.

In 2006, B. terrestris was designated an invasive alien species under the Invasive Alien Species Act in Japan. According to this law, farmers wishing to use B. terrestris for pollination must be issued permits from the responsible minister. The permit requires that farmers prevent B. terrestris from escaping from greenhouses by using covering nets, and that farmers kill B. terrestris colonies after use. After enforcement of this law, the number of B. ignitus colonies used for pollination increased because farmers do not need permission to use this species (Yoneda et al., 2008). The use of B. ignitus in Japan could be problematic because B. ignitus is not indigenous to Hokkaido, which is biogeographically separated from the other Japanese islands by the Blakiston Line. The situation with B. ignitus and B. h. sapporoensis is further complicated, as both species are indigenous to Korea and China (see http://www.nhm.ac.uk/research-curation/research/projects/bombus/regions.html). Bombus ignitus populations in China, Korea, and Japan have diverged due to recent bottlenecks and geographic isolation (Shao et al., 2004). In particular, Japanese B. ignitus are remarkably differentiated from the continental populations, and it is necessary to prevent homogenization of the separate gene pools when using B. ignitus as a pollinator. The same could be true for B. h. sapporoensis. If these two species are mass-reared in Korea and China by bumblebee companies and imported to Japan, then the Japanese gene pools will be at risk of homogenization. It is necessary to evaluate the magnitude of intraspecific genetic divergence across nations and that of hybrid production between species and/or subspecies pairs to prevent population declines of native species due to artificial species introductions.

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