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Original article

Nest density, genetic structure, and triploid workers in exotic *Bombus terrestris* populations colonized Japan*

Teruyoshi NAGAMITSU[1], Hiroki YAMAGISHI[2]

[1] Hokkaido Research Center, Forestry and Forest Products Research Institute (FFPRI), Sapporo 062-8516, Japan
[2] Laboratory of Ecology and Genetics, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

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Abstract – A commercialized pollinator introduced from Europe, *Bombus terrestris*, has colonized Japan. We investigated nest density and genetic structure in two sites based on worker genotypes at 12 microsatellite loci. We confirmed that five workers were triploids using multilocus genotypes and flow cytometry, indicating that queens mated with diploid males and produced triploid workers. The inbreeding coefficient of diploid workers representing individual colonies was significantly positive (F_{IS} = 0.048) in a site where triploids were found. Genetic diversity in the sites was as high as that in native regions in Europe, and genetic differentiation between the sites was low (F_{ST} = 0.006). The maximum distance between sampling locations of full-sib worker pairs indicated that the radius of a foraging range was at least 782 m. The estimates of nest density were 31 and 89 km^{-2} in the two sites, suggesting that the nest density in a colonized region can be higher than that in the native regions.

nest density / foraging range / genetic diversity / inbreeding coefficient / triploid female

1. INTRODUCTION

Biological invasion of exotic social insects of Hymenoptera, such as ants, wasps, and bees, is a major threat to biodiversity in native ecosystems (Chapman and Bourke, 2001). The commercialized European bumble bee, *Bombus terrestris* (L.), has been exported to various regions in the world including Japan, where commercial colonies have been introduced for the pollination of tomato crops in greenhouses since 1991. The import of *B. terrestris* colonies to Japan has been increasing, and approximately 70 000 colonies were used in 2004. (Kunitake and Goka, 2006). Queens escaping from greenhouses have been found after the introduction, and feral colonies were first discovered from Hokkaido, northern Japan, in 1996 (Nakajima et al., 2004). In some agricultural landscapes, *B. terrestris* has become the most abundant bumble bee species (Inari et al., 2005; Nagamitsu et al., 2007b). Floral resources and nest sites are overlapped between *B. terrestris* and native bumble bee species (Matsumura et al., 2004; Inoue et al., 2007). Such exotic bees potentially affect native bees and plants pollinated by them (Goulson, 2003). The potential impacts of *B. terrestris* on Japanese native communities of bees and plants include the transmission of parasites and pathogens (Goka et al., 2006), competition for nesting and floral resources (Nagamitsu et al., 2007a), and alteration of pollination systems (Kenta et al., 2007).

In the initial phase of invasion, a feral *B. terrestris* population in the Australian island of Tasmania was so inbred that it produced diploid males, probably due to mating between full-sibs of a single founder colony (Buttermore et al., 1998). Bumble bees are

Corresponding author: Dr Teruyoshi Nagamitsu, nagamit@ffpri.affrc.go.jp
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haplodiploids and have the complementary sex determination in a single locus with many alleles (Duchateau et al., 1994); females develop when the sex locus is heterozygous (X^aX^b), and males develop when it is hemizygous (X^a) or homozygous (X^aX^a). Inbreeding, such as mating between full-sibs, produces diploids homozygous in the sex locus, which are diploid males. Normal females and males develop from fertilized (diploid) and unfertilized (haploid) eggs, respectively. Thus, diploid males develop from eggs that would normally become workers or queens, and can reduce the colony growth and reproductive output. Experimentally produced diploid males sired fewer viable offspring than haploid males (Duchateau and Marien, 1995). The offspring derived from mating between normal queens and diploid males were sterile triploids that inherit haploid genome of the queens and diploid genome of the males (Ayabe et al., 2004). Therefore, the production of diploid males is a cost of inbreeding in *B. terrestris*, which may inhibit colonization and spread during invasion (Hingston, 2005). However, *B. terrestris* has spread into native vegetation in Tasmania (Hingston, 2006), and is able to produce queens and increase its population (Hingston et al., 2006).

In a colonized region, genetic diversity in founder populations may be low due to its small effective population size N_e if a few founders colonized. In bumble bees, small populations of rare species in fragmented habitats showed lower genetic diversity and higher genetic differentiation (Darvill et al., 2006; Ellis et al., 2006) than large populations of common species (Widmer and Schmid-Hempel, 1999). In *B. terrestris*, Canary Island populations in the Atlantic Ocean had lower genetic diversity than mainland populations in the European Continent, which reflects bottlenecks during colonization to the islands from the continent (Estoup et al., 1996; Widmer et al., 1998). Similarly, during colonization of invasive *B. terrestris* in Tasmania, a very small founder population produced a bottleneck, thus reducing genetic diversity in the resulting population even after they have become abundant (Schmid-Hempel et al., 2007). On the other hand, regular import of commercial colonies and subsequent escapes of queens can increase genetic diversity.

An approach to access the degree of colonization of invasive bumble bees is estimation of nest density in the fields. Nest density can be obtained from the number of full-sib groups among foraging workers and the area of their foraging range around individual nests, in which the full-sib groups are estimated from microsatellite genotypes (Chapman et al., 2003; Darvill et al., 2004; Knight et al., 2005). Because of monandry in *B. terrestris* (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000), all workers of the same nest are full-sibs, and the number of full-sib groups is identical to the number of nests. Nests assigned from full-sib grouping of workers exist within the radii of the foraging range from locations where the workers are sampled. The radii of the foraging range estimated in native *B. terrestris* were 625 and 758 m, and the estimates of its nest density were 13 and 29 km^{-2} in the UK (Darvill et al., 2004; Knight et al., 2005).

In this study, we aimed to estimate the genetic structure, foraging range, and nest density based on workers sampled in two sites where *B. terrestris* had colonized. In addition to these estimations, we reported triploid workers found in one of the two sites. Using multilocus microsatellite genotypes, we identified full-sib groups (colonies) among the workers and examined the genetic structure of their populations. According to the radius of the foraging range estimated from distances between sampling locations of the full-sib pairs, we obtained the nest density from the estimates of the number of colonies and the area where their nests can exist. Finally, we compared the nest density in the study sites with those reported from native regions.

2. MATERIALS AND METHODS

2.1. Worker sampling

In the Chitose River basin, Hokkaido, Japan, we selected two sites, A (Shimamatsu-kita, 42°55.7'N, 141°34.5'E, 22 m altitude) and B (Kamiyamaguchi-kita, 42°54.5'N, 141°37.6'E, 10 m altitude), which

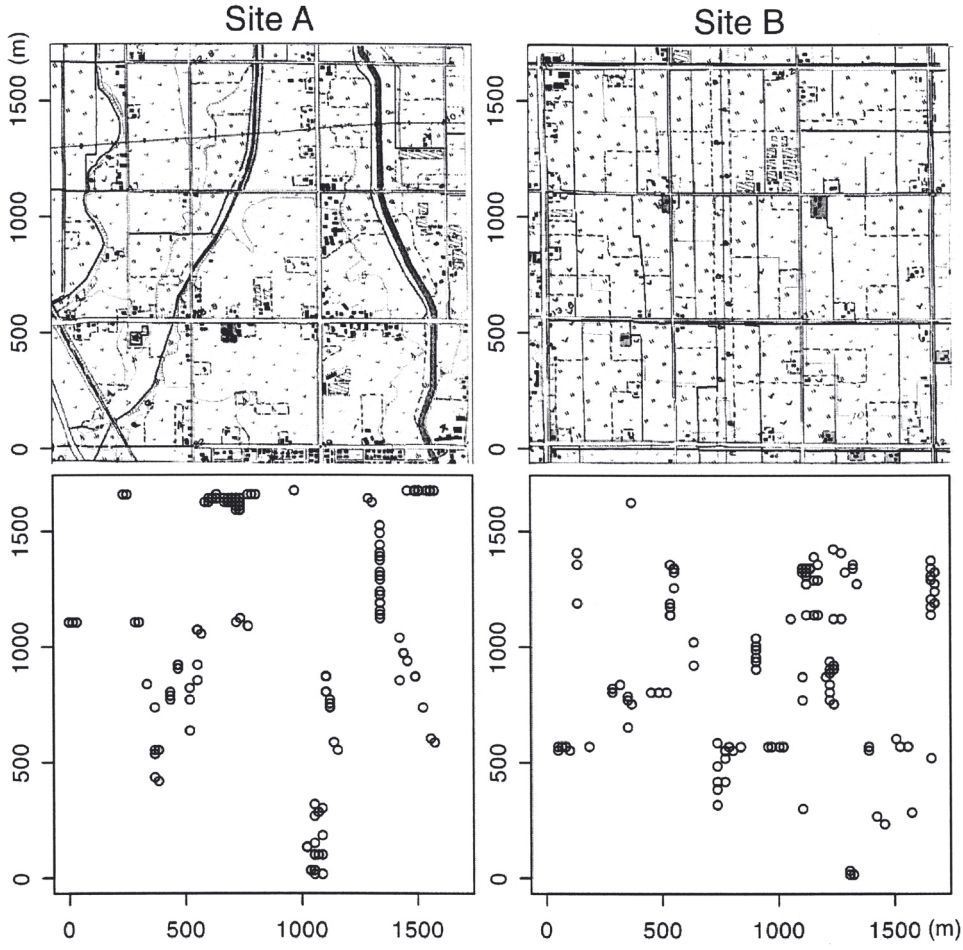

Figure 1. Maps (upper panels) and locations (circles in lower panels) where *Bombus terrestris* workers were sampled in sites A and B.

were 4.6 km apart from each other. Each 2.8 km^2 site was a square with sides 1.675 km long in an agricultural landscape covered with farms, paddy fields, windbreak forests, greenhouses, and residential buildings (Fig. 1).

During July and August in 2005, we collected *B. terrestris* workers that were flying or visiting flowers, e.g., *Barbarea vulgaris*, *Hydrangea paniculata*, *Rubus parvifolius*, *Rudbeckia laciniata*, *Solidago gigantea*, *Taraxacum officinale*, *Trifolium pratense*, and *T. repens*, at various locations along roads and rivers as well as at gardens, pastures, orchards, and forests within the two sites (Fig. 1). We collected workers using hand nets and preserved the individual workers immediately in 1 mL of 99.5% ethanol in 1.5 mL microcentrifuge tubes. In our laboratory, we measured the head width (mm) of the workers (Nagamitsu et al., 2007b) using an electric digital caliper (CD-20, Mitsutoyo Coporation, Kawasaki) to compare body size between diploid and triploid workers.

2.2. Microsatellite genotyping

Total DNA was extracted from flight muscle in the thorax of preserved workers using Chelex 100 Resin (BioRad, Hercules) (Walsh et al., 1991). A piece (5–10 mm^3) of the muscle was squashed in 0.2 mL of a water solution of 20% Chelex 100 Resin in a 1.5 mL micro tube and then heated at 98 °C for 5 min. The tube was placed on ice to cool the

solution immediately and then centrifuged at 15 000 rpm for 5 min. Finally, 5 μL of the supernatant was added into 95 μL of TE buffer (10 mM Tris-HCl and 10 mM EDTA, pH 7.4), and 100 μL of a DNA solution available for templates in polymerase chain reaction (PCR) was obtained.

The genotypes of the workers were determined from 12 microsatellite loci: B10, B11, B121, B124, and B126 (Estoup et al., 1995); B96, B100, B118, and B132 (Estoup et al., 1996); BT02, BT10, and BT22 (Funk et al., 2006). PCR was performed for each locus separately in 15 μL of a mixture containing 1 μL of the template DNA solution, 1.2 mM $MgSO_4$, 0.2 mM each dNTP, 0.2 μM each primer, and one unit of KOD plus DNA polymerase in KOD buffer (Toyobo, Tokyo) using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City) programmed for 2 min at 94 °C followed by 30 cycles of 15 s at 94 °C, 30 s at an annealing temperature optimized to each primer set, and 45 s at 68 °C. The length of the PCR products was measured using an ABI PRISM 3100-Avant Genetic Analyzer and GENESCAN Analysis Software (Applied Biosystems).

2.3. Ploidy measurement

Some workers were found to have three alleles at some loci. To determine the ploidy of the workers in comparison with diploid workers and haploid males that were collected from the same site, a Partec PA cytometer (Partec, Münster) was used. The five putative triploid workers stored in 99.5% ethanol and fresh bodies of five diploid workers and five haploid males were used for the measurement. To prevent contamination with endodiploidized cells (Aron et al., 2005), cells in the heads were examined. Tissues in the head of each individual were chopped using a razor blade in 0.3 mL of an Extraction Buffer (Partec) solution in a plastic petri dish. Next, CyStain UV Precise P Kit with DAPI (Partec) was added to the solution, and the tissues were stained for 5 min. After the solution had been filtered using a CellTrics Filter (Partec) to separate the cells from the tissues, flow cytometry analysis was performed on the solution with separated cells using the Partec PA cytometer. Finally, the ploidy was determined on the basis of the intensity of fluorescence signals at the peak of cell counts according to the manufacture's recommendations.

2.4. Full-sib estimation and genetic structure analysis

Among the diploid workers sampled from each site, we estimated the full-sib groups from the genotypes determined at the 12 loci using COLONY1.2 (Wang, 2004). The rates of allele dropout and genotyping error were set to 0.01 because the estimated rate of genotyping error per locus ranged from 0.0025 to 0.02 in microsatellites (Hoffman and Amos, 2005). Because a single monandrous queen founds her colony in a nest in *B. terrestris* (Estoup et al., 1995; Schmid-Hempel and Schmid-Hempel, 2000), we regarded each full-sib group as a single colony in a nest.

We randomly selected a single diploid worker from every colony and obtained a population of workers that belonged to different colonies in each site. We tested the genotypic linkage disequilibrium at each locus in each population and the deviation from Hardy-Weinberg equilibrium at each locus on the basis of F_{IT} using FSTAT 2.9.3 (Goudet, 2002). Sequential Bonferroni corrections were applied to keep a significance level ($P = 0.05$).

We examined the genetic structure at each locus in terms of the number of alleles, the Nei's (1987) estimator of expected heterozygosity H_e within each population as well as the Weir and Cockerham's (1984) estimators of inbreeding coefficient F_{IS} within each population and genetic differentiation F_{ST} between populations in F statistics (Wright, 1951). We tested deviations from zero in F_{IS} and F_{ST} at each locus and over all loci by means of 1000 randomizations over individuals using FSTAT 2.9.3.

Bottlenecks, recent reductions in effective population size N_e, were evaluated using BOTTLENECK 1.2.02 (Piry et al., 1999). In bottlenecked populations, heterozygosity will exceed that expected from the mutation-drift equilibrium. The expected equilibrium heterozygosity depends on mutation models. In three models, i.e., the infinite allele model (IAM), the two-phase mutation model (TPM with 70% multi-step changes), and the stepwise mutation model (SMM), we examined the excess of heterozygosity over loci using the Wilcoxon signed-rank test.

2.5. Foraging range and nest density estimation

Because full-sib workers belong to the same nest, multiple nest mate workers can be sampled

within the foraging range of the nest. Thus, distances between sampling locations of full-sib pairs are less than twice as long as the radius of the foraging range of individual nests. We obtained the frequency distribution of the distances between sampling locations of full-sib pairs and the relative frequency of full-sib pairs among all pairs of sampled workers in each distance class.

The nests of sampled workers can exist within the estimated radii of the foraging range from sampling locations. The area A (km^2) where the nests could exist was calculated in each site. The number of nests in each site was estimated assuming that the number of workers sampled per nest followed a Poisson distribution and that nests from which no worker was sampled could not be observed (Darvill et al., 2004; Knight et al., 2005). Thus, the expected value of the Poisson distribution is identical to the mean number of workers sampled from all nests, including ones from which no worker is sampled. The number of workers sampled per nest follows a Poisson distribution:

$$P(x, \lambda) = \frac{e^{-\lambda}\lambda^x}{x!} \quad (1)$$

where $P(x, \lambda)$ is the probability that the number of workers sampled per nest is $x = \{0, 1, 2, 3, \ldots\}$, and λ is the mean number of workers sampled from all nests. The likelihood equation was defined as a product of the probability that more than zero workers were sampled:

$$L(\lambda) = \prod_{x=1}^{m} \left\{ \frac{e^{-\lambda}\lambda^x}{x!} \Big/ \left(1 - \frac{e^{-\lambda}\lambda^0}{0!}\right) \right\}^{n_x} \quad (2)$$

where n_x is the number of nests from which $x = \{1, 2, 3, \ldots, \mathrm{m}\}$ workers were sampled. The maximum likelihood estimate of λ and its 95% confidence interval (a range of λ with the cumulative likelihood density between 0.025 and 0.975) were obtained according to the likelihood defined above. The likelihood maximization was performed using R 2.4.1 (R Development Core Team, 2006). The estimated number of nests N was calculated from an equation:

$$N = \sum_{x=1}^{m} n_x \Big/ \left(1 - \frac{e^{-\hat{\lambda}}\hat{\lambda}^0}{0!}\right) \quad (3)$$

where $\hat{\lambda}$ is the maximum likelihood estimate of λ. The nest density was obtained from N/A in each site, where A is the area (km^2) in which nests could exist.

3. RESULTS

3.1. Triploid workers

We sampled a total of 127 and 116 workers from various locations in sites A and B, respectively (Fig. 1). Five of the 127 workers in site A had three alleles at 4–6 of the 12 loci. In site B, there were no such putative triploid workers. Flow cytometry demonstrated that the DNA contents of the five putative triploids were 1.5 times as large as those of diploid workers and three times as large as those of haploid males (Fig. 2). There were more noises in the triploid workers stored in 99.5% ethanol (Fig. 2a) than in fresh bodies of the diploid workers and the haploid males (Fig. 2b, c). These noises seemed to be signals from fragmented DNA from broken cells that had been produced during storage. The head width of the triploid workers (3.51 ± 0.22 mm, mean ± standard deviation, $n = 5$) was significantly smaller than that of diploid workers in site A (3.87 ± 0.27 mm, $n = 122$; Mann-Whitney test, $P = 0.007$). Full-sib triploids should share two alleles in every locus derived from diploid males that sired them (Ayabe et al., 2004). According to the criterion, we identified four full-sib groups among the five triploids. One of the four full-sib groups consisted of two workers sampled at locations 213 m distant from each other.

3.2. Full-sib diploid workers and their genetic structure

Among 122 and 116 diploid workers sampled from sites A and B, we identified 97 and 107 full-sib groups (colonies), respectively (Tab. I). We sampled a single diploid worker from every colony and then obtained two populations of diploid workers that belonged to different colonies in sites A and B. No pairs of loci showed significant genotypic linkage disequilibrium when we tested them both within each population and across the two populations (randomization test, $P > 0.05$). We found significant deviations from Hardy-Weinberg equilibrium at three loci, B10, B96, and B126, on the basis of F_{IT} ($P < 0.031$).

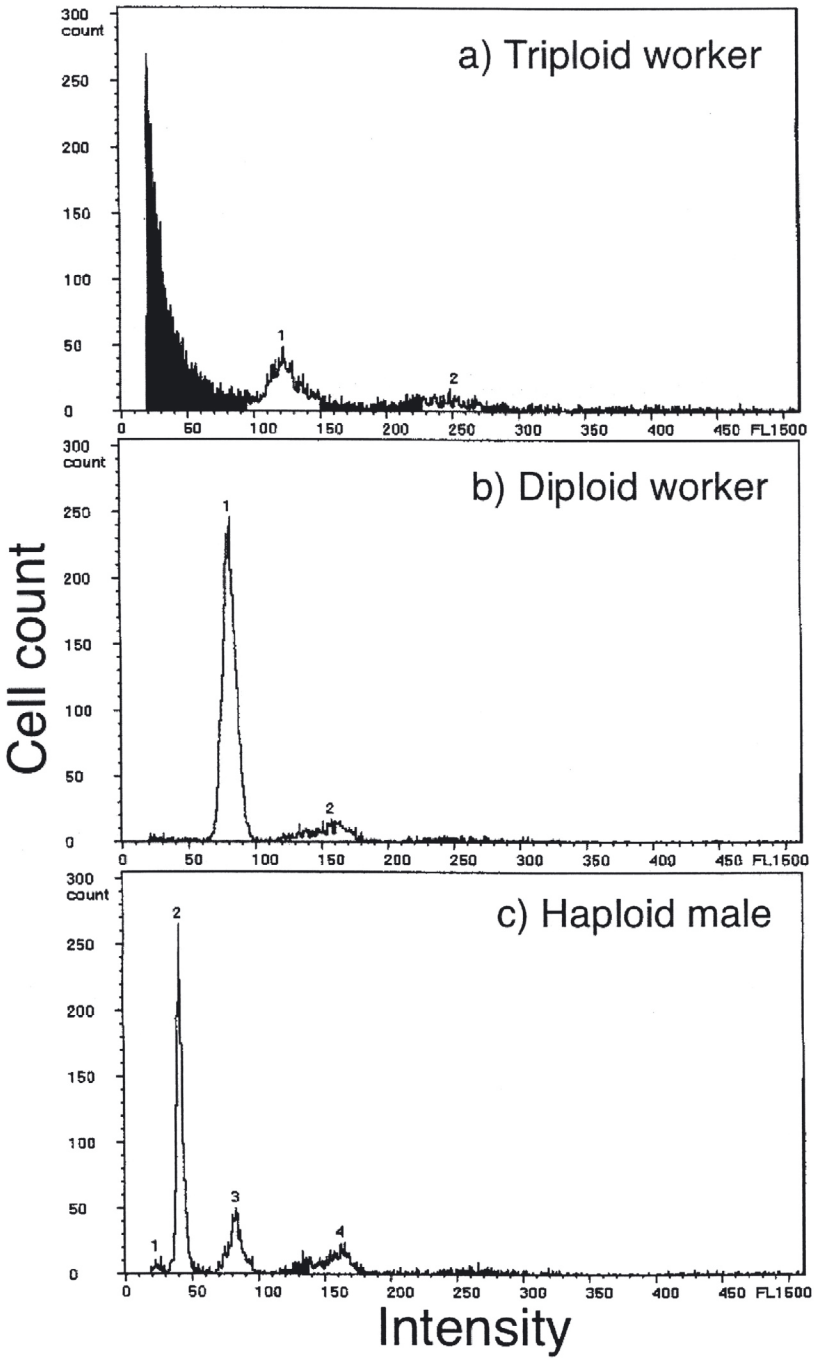

Figure 2. Cell counts with the intensity of fluorescence signals measured using flow cytometry in a putative triploid worker (a), a diploid worker (b), and a haploid male (c) of *Bombus terrestris*.

Table I. Frequency distributions of the observed and expected number of *Bombus terrestris* diploid workers sampled per full-sib group in sites A and B.

Number of full-sib groups	No. of workers sampled per full-sib group					Total
	0	1	2	3	> 3	
Site A						
Observed	NA	73	23	1	0	97
Expected	157.4	75.6	18.1	2.9	0.4	254.4
Site B						
Observed	NA	98	9	0	0	107
Expected	616.7	98.7	7.9	0.4	0.0	723.7

The mean number of alleles per locus was higher in site B (11.5) than in site A (10.5)(Tab. II). The expected heterozygosity H_e averaged over 12 loci was also higher in site B ($H_e = 0.780$) than in site A ($H_e = 0.751$) but was not significantly different between the two sites (Wilcoxon signed-rank test, $n = 12$, $P = 0.071$). The inbreeding coefficient F_{IS} within each population was significantly positive at three loci, B10, B96, and B126, in both sites A and B (randomization test, $P < 0.042$). When all loci were considered, F_{IS} was significantly positive ($F_{IS} = 0.048$) in site A ($P < 0.002$) but was not significantly different from zero in site B ($P = 0.138$). The genetic differentiation F_{ST} between the populations was significantly positive at five loci ($P < 0.034$) and significantly positive ($F_{ST} = 0.006$) when all loci were concerned ($P < 0.001$).

Evidence for bottlenecks, as indicated by an excess of heterozygosity on the basis of mutation-drift equilibrium, depended on the assumed mutation models in each population. There was a significant excess of heterozygosity in the IAM (Wilcoxon signed-rank test, $n = 12$, $P < 0.001$), although there was a significant deficit of heterozygosity in the SMM ($P < 0.021$). In the TPM, there was neither significant excess nor deficit of heterozygosity ($P > 0.088$).

3.3. Foraging range and nest density

In both sites A and B, there were 1 and 32 colonies with three and two sampled workers, respectively (Tab. I). The mean, median, and maximum distances between sampling locations of the 35 full-sib pairs were 529, 437, and 1564 m, respectively. Thus, the radius of the foraging range was at least 782 m, half of the maximum distance between sampling locations of the full-sib pairs. The number and relative frequency of full-sib pairs were the largest in the distance class from 200 to 400 m and tended to decrease with increasing distance in the range from 200 to 1600 m (Fig. 3).

The areas within the estimated radii of foraging range (782 m) from sampling locations, where nests of sampled workers could exist, were 8.14 and 8.11 km^2 in sites A and B, respectively. The maximum likelihood estimates with their 95% confidence intervals of the mean number of workers sampled per nest in the Poisson distributions were 0.48 [0.32, 0.68] and 0.16 [0.08, 0.30] in sites A and B, respectively. Thus, the estimated numbers of nests were 254.4 [196.6, 354.2] and 723.7 [412.8, 1391.7] in sites A and B, respectively (Tab. I). The observed and expected numbers of nests from which single and multiple workers were sampled did not differ significantly (χ^2 test, $df = 1$, $P > 0.789$). Therefore, the estimates with their 95% confidence intervals of nest density were 31.3 [24.2, 43.5] and 89.2 [50.9, 171.5] km^{-2} in sites A and B, respectively.

4. DISCUSSION

4.1. Triploids, inbreeding, and genetic diversity

We demonstrated the presence of triploid *B. terrestris* workers of multiple colonies in the field. Both microsatellite genotyping and flow cytometry confirmed the triploidy of five

Table II. Number of alleles, expected heterozygosity H_e, and inbreeding coefficient F_{IS} within each population of *Bombus terrestris* diploid workers representing individual colonies in sites A and B as well as genetic differentiation F_{ST} between the populations at 12 microsatellite loci and over these loci. Asterisks show significant differences ($P < 0.05$) from zero, and "ns" shows that the difference is not significant.

Locus	No. of alleles		H_e		F_{IS}		F_{ST}
	A	B	A	B	A	B	
B10	18	19	0.903	0.933	0.064 *	0.028 *	0.007 *
B11	10	10	0.817	0.824	0.078 ns	–0.055 ns	0.012 *
B96	5	5	0.681	0.642	0.107 *	0.054 *	0.004 ns
B124	10	14	0.845	0.837	0.036 ns	0.006 ns	0.002 ns
B126	10	12	0.827	0.866	0.153 *	0.071 *	0.005 ns
B121	3	3	0.203	0.322	–0.015 ns	0.041 ns	0.013 *
B100	9	9	0.682	0.744	0.063 ns	–0.018 ns	0.004 ns
B118	8	9	0.686	0.742	0.083 ns	–0.021 ns	0.010 *
B132	11	11	0.849	0.847	–0.008 ns	0.018 ns	–0.003 ns
BT02	14	15	0.771	0.846	–0.030 ns	0.017 ns	0.021 *
BT10	14	17	0.866	0.852	–0.024 ns	0.068 ns	–0.001 ns
BT22	14	14	0.884	0.901	0.044 ns	–0.027 ns	0.001 ns
Overall	10.5	11.5	0.751	0.780	0.048 *	0.014 ns	0.006 *

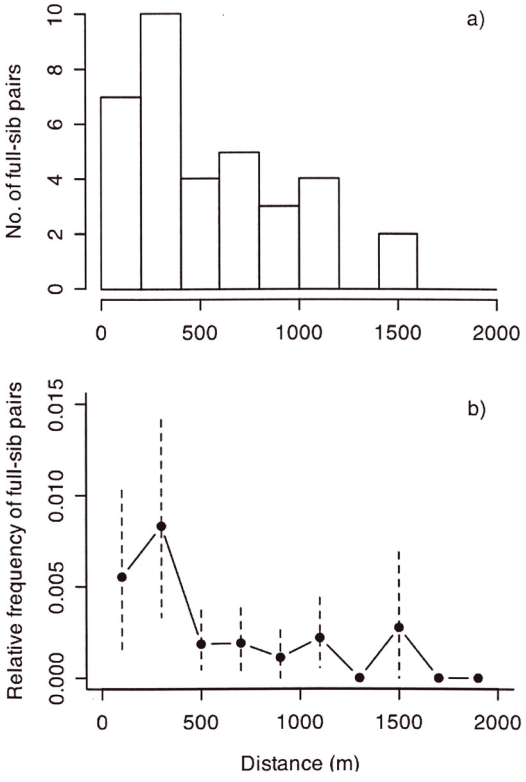

Figure 3. Frequency distributions of the number (a) and relative frequency (b) of 35 full-sib pairs among all pairs of sampled *Bombus terrestris* workers in each class of distances between their sampling locations. Dashed bars show 95% confidence intervals of the relative frequency in each distance class.

wild workers, although the number of chromosomes was not examined because pupae were necessary to obtain mitotic cells in which chromosomes were observable. The significantly smaller body size of the triploid workers than that of diploid ones is also consistent with a previous study, which showed that the body size decreased as the ploidy level increased in *B. terrestris* (Ayabe et al., 2004). Because a diploid queen mating with a diploid male produces triploid females (Duchateau and Marien, 1995; Ayabe et al., 2004), the presence of triploid workers suggests the production of diploid males. In a rare species, *B. muscorum*, in the UK, three out of 64 males sampled were diploid (Darvill et al., 2006). Nearly half of *B. terrestris* colonies founded by field-caught queens produced diploid males in Tasmania (Buttermore et al., 1998).

The production of diploid males suggested from triploid workers indicates inbreeding (Duchateau et al., 1994). The significantly positive inbreeding coefficient (F_{IS} = 0.048) supports the inbreeding in site A where the triploid workers were found. On the other hand, three loci, B10, B96, and B126, exhibited significantly positive F_{IS} in both sites A and B. This result suggests the presence of null alleles at the three loci, which results in an excess of apparent homozygotes (hetetrozygotes with a null allele). The three loci showed deviations from Hardy-Weinberg equilibrium in previous studies: Widmer et al. (1998) for B10, Darvill et al. (2004) for B96, and Knight et al. (2005) for B126. Thus, null alleles at the three loci may partly increase F_{IS} in both sites A and B.

The expected heterozygosity H_e in our study sites was as high as that in native regions in the European Continent according to six comparable loci: H_e = 0.800–0.900 in B11, 0.494–0.632 in B96, 0.579–0.863 in B100, 0.685–0.851 in B118, 0.272–0.459 in B121, and 0.826–0.908 in B132 (Estoup et al., 1996). There was no clear evidence for bottlenecks in our study site. Thus, genetic diversity was unlikely to decline during colonization in Japan due to escapes of multiple queens over several years. These results contrast with the findings in Tasmania, where genetic diversity was lower than that in New Zealand and Europe probably due to a very few founders arriving at Tasmania from New Zealand (Schmid-Hempel et al., 2007).

The genetic differentiation (F_{ST} = 0.006) between sites A and B was significantly positive and similar to that (F_{ST} = 0.005) among various regions in the European Continent (Estoup et al., 1996). Because several companies shipped commercial colonies to Japan, feral populations seemed to originate from multiple sources that might be established from different regional populations in Europe. Therefore, different assemblages of fugitives from the sources between the sites were likely to result in the significant genetic differentiation. However, F_{ST} in our study sites was lower than that (F_{ST} = 0.411) among isolated populations in the Canary Islands and Madeira (Widmer et al., 1998). Thus, migration of queens and males is likely to occur in the colonized region including our study sites. Statistical modeling for a spatial distribution of *B. terrestris* bees trapped in the same region indicated that fugitives from greenhouses had dispersed over 18.8 km during 1992 and 2004 (Nagamitsu et al., 2007b). These findings suggest that feral populations from different sources are connected by gene flow and have been mixed by migration in the colonized region.

4.2. Foraging range and nest density

The foraging range in *B. terrestris* has been investigated using homing experiments, mathematical modeling, and field observations by means of mark and recapture, radar tracking, and genetic markers. In homing experiments, workers were able to return to their nests from distances up to 9.8 km (Goulson and Stout, 2001). An economic model to optimize foraging efficiency predicted that foragers flying over several kilometers from their nests could gain a net energy uptake (Cresswell et al., 2000). In contrast to the larger potential of the foraging range, field observations demonstrated that a smaller foraging range was realized. In an agricultural landscape, marked workers were recaptured at distances up to 1750 m from their nests (Walther-Hellwig and

Frankl, 2000). Radar tracking for 65 foraging trips showed that the maximum distance from nests ranged from 70 to 631 m in a farmland (Osborne et al., 1999). Using genetic markers, the estimated radii of the foraging range were 625 m (Darvill et al., 2004) and 758 m (Knight et al., 2005) in rural landscapes. In our study, the radius of the foraging range was at least 782 m according to distances between sampling locations of full-sib worker pairs, of which frequency distribution showed a similar declining curve of the frequency with increasing distance in a previous study (Knight et al., 2005).

To estimate the number of nests in an area within the radius of the foraging range from sampling locations, a truncated Poisson distribution was applied to the number of workers sampled per nest. This application is reasonable when workers are sampled from all nests at the same probability. Darvill et al. (2004) and Knight et al. (2005) sampled workers from each point on a transect to estimate the number of nests around the individual sampling points. In such a case, workers of nests closer to the sampling points are more likely collected. Thus, the number of workers sampled per nest is likely to follow a negative binomial (aggregated) distribution rather than a Poisson (random) distribution. In our study, workers were sampled at various locations in an area, which might make the probability that workers were sampled from every nest similar. For this reason, the Poisson distribution fitted to the number of workers sampled per nest in our study.

The estimates of nest density in *B. terrestris* were significantly lower in site A (31 km^{-2}) than in site B (89 km^{-2}). This result is consistent with the mean number of *B. terrestris* bees collected using a window trap, 4.75 in site A and 23.00 in site B (Nagamitsu et al., 2007b). The nest density estimated in site B is significantly higher than those (13 and 29 km^{-2}) estimated in the UK among native regions (Darvill et al., 2004; Knight et al., 2005) although estimates of nest density are unavailable in the European Continent where *B. terrestris* seems to be abundant. Exotic organisms often become more abundant in colonized regions than in their native regions, which is probably due to the fact that they escape from their parasites and predators and avoid their competitors (Keane and Crawley, 2002). Social bees, in particular, *Apis mellifera*, are likely to maintain high nest density in regions where they colonized, such as Australia (Oldroyd et al., 1995) and the neotropics (Roubik, 1983), in comparison with the natural nest density in their native regions.

In our study, the relatively high estimates of nest density and genetic diversity conflicts with inbreeding suggested from triploid workers and genetic structure. A possible explanation for this conflict is low mate availability. Isolated colonies may suffer from the low mate availability within their mating range although the mating range of *B. terrestris* is unclear. During colonization to new habitats, spatial and temporal isolation is likely to occur because founders colonizing new nest sites may be spatially separated, and different conditions in the new sites may alter their reproductive timing. The evidence for inbreeding only from site A where nest density was relatively low supports this explanation because the mate availability seems to be low in nests distributed sparsely.

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Densité des nids, structure génétique et ouvrières triploïdes chez les populations de *Bombus terrestris* introduites au Japon.

***Bombus terrestris* / espèce introduite / distance de butinage / diversité génétique / génétique des populations / coefficient de consanguinité / densité de population / triploïdie**

Zusammenfassung – Nestdichte, genetische Struktur und triploide Arbeiterinnen bei

eingeführten *Bombus terrestris* Populationen in Japan. Die kommerziell aus Europa eingeführte Bestäuberart *Bombus terrestris* hat sich inzwischen über ganz Japan verbreitet und beeinflusst dadurch möglicherweise einheimische Bienen- und Pflanzenarten. In den Verbreitungsgebieten scheint die genetische Diversität geringer zu sein als in ursprünglichen Regionen, da die Gründerpopulationen meist recht klein sind. Durch Inzucht in kleinen Gründerpopulationen entstehen diploide Männchen, die bei der Paarung triploide Individuen zeugen. Trotz der Inzucht und der verringerten genetischen Variabilität vergrößern wilde Populationen ihr Verbreitungsgebiet und kommen dann teilweise recht häufig vor. Wir untersuchten die Nestdichte und die genetische Struktur bestimmter Genotypen an zwei Untersuchungsstandorten in Nordjapan auf der Basis von 12 Mikrosatelliten-Loci von sammelnden Arbeiterinnen (Abb. 1). Wir bestätigten anhand von Multilocus-Genotypanalysen, dass fünf Arbeiterinnen triploid waren (Abb. 2); deren Königinnen hatten sich vermutlich mit diploiden Männchen verpaart und danach triploide Arbeiterinnen produziert. Die Ergebnisse lassen Inzucht vermuten. Folgerichtig war der Inzuchtkoeffizient von diploiden Arbeiterinnen aus Einzelvölkern signifikant positiv ($F_{IS} = 0,048$), wenn an den Standorten triploide Arbeiterinnen gefunden wurden (Tab. II). Die genetische Diversität an diesen Standorten war genauso hoch wie in den Ursprungsregionen in Europa und die genetische Differenzierung zwischen den Standorten war gering ($F_{ST} = 0,006$; Tab. II). Diese Ergebnisse lassen vermuten, dass diese Wildpopulationen von verschiedenen Linien kommerziell eingeführter Völker abstammen und dass es durch Migration zur Vermischung verschiedener Populationen kam. Die maximale Distanz zwischen Sammelorten von Vollgeschwister-Paaren zeigt, dass der Sammelradius mindestens 782 m beträgt (Abb. 3). Die geschätzte Nestdichte von 31 und 89 km^{-2} an zwei verschiedenen Standorten (Tab. I) zeigt, dass die Dichte im neuen Verbreitungsgebiet höher sein kann als in den Ursprungsregionen. Unsere Befunde lassen vermuten, dass reduzierte Paarungsmöglichkeiten für die Inzucht in eingeschleppten *B. terrestris*-Populationen verantwortlich sind.

Nestdichte / Sammelradius / genetische Diversität / Inzuchtkoeffizient / Triploide Weibchen

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