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Effect of insect pollinator on inbreeding versus outbreeding in open pollinated strawberry seeds

Tomohiro Yanagi[a,*], Hiroto Miura[a], Sachiko Isobe[b], Nobuyuki Okuda[a], Yuichi Yoshida[c]

[a] Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa, Japan
[b] Kazusa DNA Research Institute, Kazusa-Kamatari, Kisarazu, Chiba, Japan
[c] Faculty of Agriculture, Okayama University, Okayama, Okayama, Japan

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ABSTRACT

In Japan, honey bees, bumble bees, and flies are used in strawberry production to produce normally shaped fruits. These insects may determine the inbreeding or outbreeding of strawberry flowers. Whole-genome DNA of the strawberry cultivar 'Shizutakara' showed no amplification of DNA fragments when PCR was performed with a F3H CAPS DNA marker. However, in other ordinary strawberry cultivars, amplification of DNA fragments was observed on PCR with the F3H. Thus, growing one plant of 'Shizutakara' with other cultivars and a single insect species, harvesting 'Shizutakara' fruit, and performing DNA analysis of the seeds could show whether the seed resulted from inbreeding or outbreeding, as the DNA fragments would be amplified from outbred but not from inbred seed. On this basis, differences in pollination mode among these three pollinators were analyzed. DNA analysis showed that DNA fragments could not be detected in seeds obtained by fly pollination in the greenhouse. Flies appeared to promote inbreeding. For honey bees and bumble bee, amplified DNA fragments could be detected. Some seeds pollinated by honey bees and bumble bee appeared to be of outbred origin. From these results, differences in pollination mode among insect pollinators were identified.

1. Introduction

In Japan, over 98% of strawberry growers use a high plastic tunnel greenhouse in winter. The cropping system is called "Forcing culture". In the forcing culture, insect pollinators such as honey bees (*Apis mellifera* L.), bumble bees (*Bombus terrestris* L.), and flies (*Lucilia sericata* M.) must be used to produce larger, better-shaped fruits. Honey bees are the most prominent insect pollinator in Japan (Matsuka and Sakai, 1989). Many studies have shown that the rate of malformed fruit production decreased after placing honey bees in the greenhouse (Chang et al., 2001; Paydas et al., 2000; Zaitoun et al., 2006; Ariza et al., 2012) and open fields (Free, 1968; Nye and Anderson, 1974; Svensson, 1991; Bartomeus et al., 2014; Klatt et al., 2014; Zapata et al., 2014). Bumble bees also are used in the forcing culture because of their low-temperature tolerance (Paydas et al., 2000). Zaitoun et al. (2006) and Dimou et al. (2008) reported that the use of bumble bee increased fruit yield and reduced the incidence of malformed fruit. Jaycox (1970) showed that flies are one of the insects that could pollinate strawberry flowers. The use of flies is gradually increasing in Japan because they do not sting.

Bees and flies visit strawberry flowers for different reasons. Honey bees and bumble bees collect floral nectar and pollen as foodstuff. When their worker bees collect them, they spend some time on the strawberry flower (Von Frisch, 1959). Currently, pollination is primarily by the work bees. In the case of the honey bees, they scrape pollen from anthers with their forelegs and add honey to the pollen, thereby increasing its adhesion, and store it in the pollen baskets. The honey bees rapidly transfer the pollen on its body hairs to pollen baskets on the hind legs (Thorp, 2000). And honey bees fly from flower to flower. The bumble bee hakes pollen from anthers by vibrating its thorax and collects it. Then, bumble bees compress pollen grains into pollen baskets on the hind legs like honey bees do. Parker et al. (2015) indicated that the pollens in the pollen baskets of honey bees and bumble bees had lower fertility when compared with the pollen on their body. From these observation, it is unclear that honey bees and bumble bees may promote inbreeding or outbreeding of strawberry. It could be considered that the pollen, which adheres to the honey bee's body, might have little ability to fertilize ovules. Although the fly eats floral nectar, it has no specific nest or brood to care for and never transports pollen in large loads. However, few papers indicate that flies can promote

* Corresponding author.
E-mail address: yanagi@ag.kagawa-u.ac.jp (T. Yanagi).

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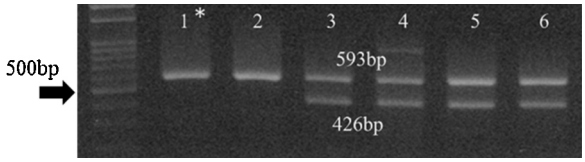

Fig. 1. Presence and absence of the DNA fragments amplified by PCR with CAPS primers of CTI1 (593 bp) and F3H (426 bp).
*1 and 2: Inbred seeds of 'Shizutakara'.
3 and 4: Outbred seeds between 'Shizutakara' × 'Tochiotome'.
5 and 6: Outbred seeds between 'Shizutakara' × 'Nyoho'.

inbreeding or outbreeding in strawberry flowers because of their sheer abundance.

Kunihisa (2011) and Kunihisa et al., (2003, 2005, 2009) developed 25 cleaved amplified polymorphic sequence (CAPS) DNA markers for the purpose of identifying cultivars of cultivated strawberry. The CAPS markers can establish the homozygosity or heterozygosity of DNA segments amplified by PCR, even though the cultivated strawberry is allo-octoploid. Testing 125 strawberry cultivars with the 25 CAPS markers revealed that no DNA fragment could be amplified by PCR with the two CAPS primers F3H and CTI2 from genomic DNAs of 'Awanatsuka', 'Kogyoku', 'Miyoshi', and 'Shizutakara' (National Agricultural Research Organization in Japan, 2007). From these results, it could be predicted that if DNA of inbred seedlings of these four cultivars was used for DNA analysis, no DNA segment would be amplified by PCR with F3H and CTI2. In contrast, DNA segments from the progeny of outbreeding between these and other cultivars would be amplified. Thus, growing one plant of 'Shizutakara' with other cultivars and a single insect species, harvesting the 'Shizutakara' fruit, and performing DNA analysis of the seeds could show whether the seed resulted from inbreeding or outbreeding as DNA fragments would be amplified from outbred seeds but not from inbred seeds.

However, few papers indicated that honey bees, bumble bees and flies could promote inbreeding or outbreeding in open-pollinated strawberry flowers. The purpose of the present study was to establish the differences in pollination success among these three pollinators.

2. Materials and methods

2.1. DNA analysis of artificially pollinated seeds in 'Shizutakara' (Exp. 1)

Three Japanese strawberry cultivars of 'Shizutakara', 'Tochiotome', and 'Nyoho' were grown in a greenhouse in the Faculty of Agriculture, Kagawa University. To produce inbred seeds of 'Shizutakara', a flower was covered with a paper bag just a few days before anticipated blooming. Opened flowers were exposed, anthers were tapped with a paintbrush, which was then gently applied to the pistils of the same flower, and flowers were again covered with the bag. To produce outbred seeds in 'Shizutakara', all stamens of flowers a few days before blooming were manually removed with forceps. The flowers were then sprayed with water to remove pollen and covered with a paper bag. Stamens of 'Tochiotome' and 'Nyoho' were harvested from flowers a few days before blooming and placed in a Petri dish to dry for one or two days. The emasculated flowers of 'Shizutakara' were then pollinated with 'Tochiotome' or 'Nyoho' pollen using a paintbrush and again covered with bags. Mature seeds were collected with forceps from mature fruits of 'Shizutakara' and used for DNA extraction.

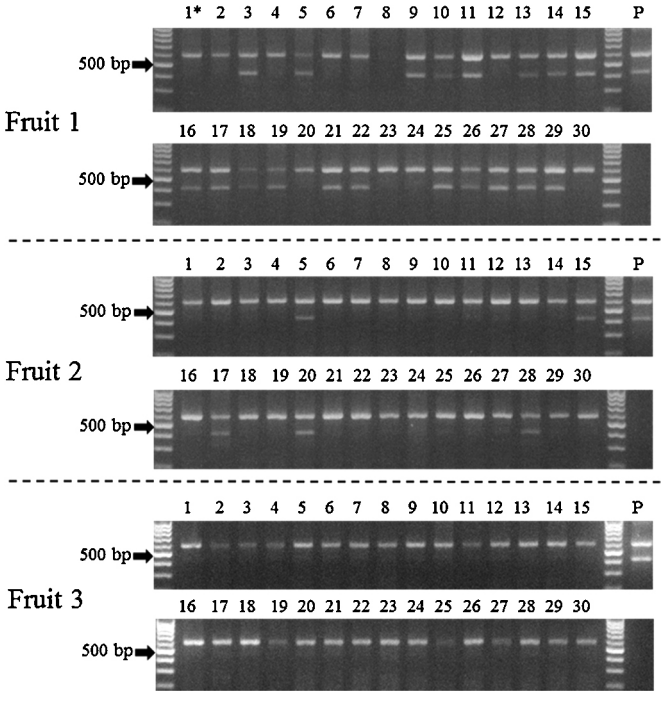

Fig. 2. The presence and absence of DNA fragments amplified by PCR with CTI1 (593 bp) and F3H (426 bp) from DNA extracted from seeds of 'Shizutakara'. The seeds were obtained from the fruits in the greenhouse of Kagawa University (KU) that had been rearing honey bees.
*1 to 30: Seeds were obtained from one fruit.
P: Seeds were obtained from fruit of 'Shizutakara' artificially pollinated by 'Nyoho'.
*Fruit 1, 2 and 3 denote seeds obtained from different fruits.

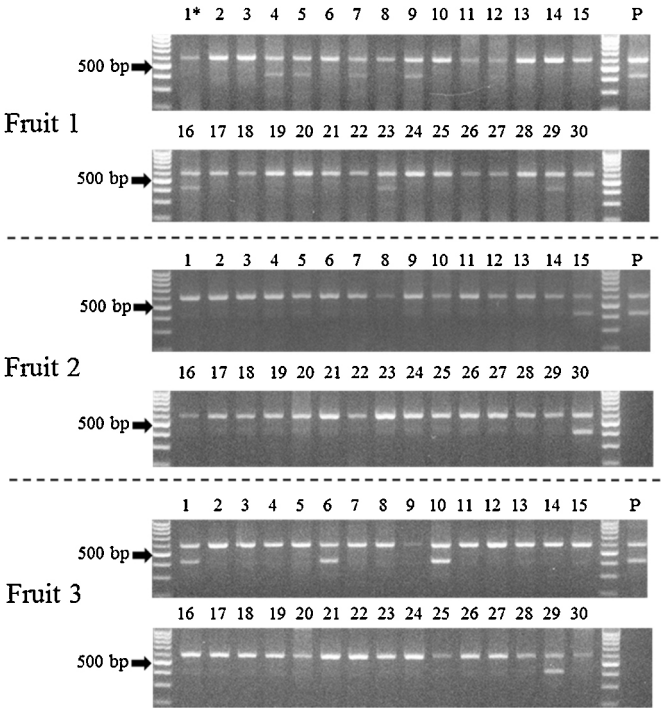

Fig. 3. The presence and absence of DNA fragments amplified by PCR with CTI1 (593 bp) and F3H (426 bp) from DNA extracted from seeds of 'Shizutakara'. The seeds were obtained from the fruits in the greenhouse in Kagawa Prefecture (KP) that had been rearing bumble bees.
*1 to 30: Seeds were obtained from one fruit.
P: Seeds were obtained from fruit of 'Shizutakara' artificially pollinated by 'Nyoho'.
** Fruit 1, 2 and 3 denote seeds obtained from different fruits.