Effects of Human Disturbance of Forest on Reproduction of a Heterodichogamous Maple, *Acer mono*

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Introduction

There is concern about the effect of human disturbance of forests on trees' reproductive success, which underpins forest ecosystems. To predict that effect, it is necessary to investigate and analyze factors affecting plant reproduction. One of the factors, local population density, which is generally altered by anthropogenic forest use, is particularly important. Many studies report low population density causing low pollination success due to there being less visitation of pollinators to flowering plants (Kunin 1997, Ghazoul and McLeish 2001, Wagenius 2006), fewer mating individuals, and inbreeding depression due to the receipt of low-quality pollen (Bosch and Waser 1999). These negative effects on pollination efficiency reduce seed set (House 1992, Aizen and Feinsinger 1994, , Cascante et al. 2002). However, several studies have showed positive effects of low population density on other reproductive stages, such as low seed predation or fungal attack (Cascante et al. 2002, Fujimori et al. 2006).

In addition to population density, other factors such as tree size may have large effect on reproduction. Size-dependent resource allocation, whereby larger plants can invest more resources in fruits and seeds, has been observed in several plant species (Ohlson 1988, de Jong and Klinkhamer 1989). Such seeds may have higher levels of soundness. Since larger plants generally have larger floral displays, they will attract more pollinator insects (Klinkhamer et al. 1989), and may have better pollination efficiency.

Many plant species of a wide variety of taxa have dichogamous flowering systems. Sex expression is also an important reproductive factor for these species. For instance, in heterodichogamous species, which have a mixture of protogynous (stigmas are receptive before pollen is shed) and protandrous (pollen is shed before stigmas are receptive) mating types in a population (Renner 2001), resource investment to fruit maturation was higher in protogynous types than protandrous types (Asai 2000, Sato 2002). It is thought that heterodichogamous plants have reciprocal mating among different sex morph types (Gleeson 1982, Dommee et al. 1990, Kimura et al. 2003) to avoid self-pollination (Cruden and Hermann-Parker 1977) and reduce self-interference (Lloyd and Webb 1986). If such pollination systems are common, individuals are limited to mating with others of reciprocal sex morph, and their pollination efficiency will be more affected by spatial distribution of the reciprocal sex morph type rather than by the overall population density.

Thus, when we consider effect of human disturbance of forest on reproduction of trees, we should assess the effect of gradient of local population on reproductive success of trees together with the effect of other factors mentioned above. However, reproductive consequences of negative or positive impact of those factors are not yet well known, especially for trees of temperate forests. Therefore, we investigate the whole

fruiting processes of *A. mono* Maxim. var. marmoratum (Nichols.) Hara f. dissectum (Wesmael) Rehder, which is a temperate forest component. This species is known to have an insect pollination system and heterodichogamous flowers as other *Acer* species. Our objective of this study is to clarify which stages of the fruiting process are affected by local population density, sex morph or individual tree size.

Species

Acer mono Maxim. var. marmoratum (Nichols.) Hara f. dissectum (Wesmael) Rehder (hereafter *A. mono*) is one of main canopy component trees in cool temperate deciduous forests in Japan. Inflorescences commonly form a compound umbel with two types of small flowers. One is a male flower with dehisced anthers and no pistil or an atrophied (sterile) pistil. The other is a female flower with indehiscent anthers and a healthy (fertile) pistil. In natural populations, a few trees have only inflorescences with male flowers and most trees have one of three types of inflorescence: (1) female flowers bloom before male flowers (female-male), (2) male flowers bloom before female flowers (male-female), or (3) male flowers bloom before and after female flowers (male-female-male). In this paper, we term (1) the protogynous (PG) type, and (2) and (3) the protandrous (PA) type. In most trees, the blooming period of each type of flower is synchronized at the individual tree level (Shibata M. personal observation). Thus, each tree has generally a male phase and a female phase in a flowering season. Various types of insects, such as flies, hoverflies and small solitary bees (Halictidae, Andrenidae) visit the flowers of this species (Matsui 1991). Thus, this species is a generalist plant; i.e. pollinated by several or many species from different taxa.

Study site

The study was carried out at Ogawa Forest Reserve (OFR) and neighboring fragmented forest, located in the southern part of the Abukuma Mountains, central Japan. The core area of OFR (ca. 100 ha) is an old-growth temperate deciduous forest. It is known that the land surrounding OFR has suffered human disturbance such as forest burning in the past (Suzuki 2002). Although OFR has been protected for at least 80 years, most of the edge area of this forest reserve is secondary forest likely to have been affected by human disturbance. Species composition and structure in the fragmented forest areas are similar to OFR (Masaki T. unpublished). The fragmented forest areas also have both old-growth and secondary forest stands. Information about the spatial location of OFR and the neighboring fragmented forest areas is shown in Isagi et al. (2007) and Fujimori et al. (2006)

Methods

Mapping of reproductive adult trees

We marked all flowering trees of *A. mono* as adult trees in a 6 ha plot of OFR and the fragmented forest areas in 2002. Canopy condition (canopy, suppressed, in gap, or gap edge), sex morph, and DBH of each tree were recorded. In order to determine the sex morph (PA or PG) of each tree, several inflorescences were collected in mid flowering season, and the condition of male and female flowers (bud, flowering, wilted, or dead) in the inflorescences was observed to determine the sexual sequence of flowering.

Since forest management differed between OFR and the fragmented forest, there was a certain degree

of gradient of local population density. The geographical position of each tree was recorded on a map of the area. Based on this map, sample trees from sites of different local population densities were selected. For each sample tree, the average distance from the nearest five adults was measured as an index of local population density. The average distance from the nearest five adults was measured in two ways: DIS-N, distance from the nearest adults irrespective of sex morph, and DIS-RS, distance from the nearest adults that have the reciprocal sex morph (for instance, the distance from the nearest five PG trees was measured for PA trees).

Comparing seed demography

In order to compare reproductive performance between canopy trees with different local population density, individual tree size, and sex morph, we sampled seeds from 17 trees in 2002, 23 trees in 2003, and 40 trees in 2005. Most seeds were collected directly from branches just before the seed fall season (late October). The number of sampled seeds was from 100 to 500 for each tree. All seeds were cut and their kernel condition was examined, categorizing them according to condition as empty, subjected to predation by insects, decayed, or sound. Percentages of these seed conditions were calculated for each tree. Other than these parameters, seed size and percentage of immature seed fall were measured in 2003 and 2005, respectively. Average kernel weight of a sound seed as an index of seed size was measured for 12 of 23 sample trees in 2003. Percentage of immature seed fall was estimated for 30 of 40 trees in 2005.

Factors affecting seed production were analyzed by multiple regression analysis. The explaining variables were 3 factors: DIS-N or DIS-RS as an index of local population density, DBH as individual tree size, and type of sex morph. DIS-N and DIS-RS were transformed to log scales. The dependent variables were 6 demographic parameters: percentage of immature seed fall, empty seeds, seeds subjected to predation by insects, decayed seeds, sound seeds, and seed kernel weight. For the percentage of empty seeds, seeds subjected to predation by insects, decayed seeds, and sound seeds, data were pooled among the 3 observation years to enlarge sample size and reveal overall trends including annual variation of seed crop.

Results

We constructed two multiple regression models, one had DIS-N, DBH, and sex morph for the explaining variables, and the other had DIS-RS, DBH, and sex morph. The latter had a lower value of AIC (Akaike's Information Criterion) than the former for all the 6 dependent variables, suggesting that DIS-RS was a clearer index than DIS-N for the effect of local population density.

Distance from conspecific adults had a significant positive correlation with the percentage of immature seed fall and empty seeds (Tab. 1), but a negative correlation with the percentage of seeds subjected to predation and decayed seeds. Distance from conspecific adults did not have significant correlation with the percentage of seed soundness. However, its positive effect on seed kernel weight was marginally significant. Sex morph was only related to immature seed fall. Trees of PG type had a significantly lower percentage of immature seed fall than trees of PA type (Tab. 1). No effect was detected for size of individual trees (Tab. 1). These trends in the explaining variables were the same for the two models.

Discussion

Factors affecting pollination efficiency

The positive effect of the distance of conspecific adults on the percentage of immature seed fall and empty seeds (Tab. 1) suggested pollination efficiency increasing with higher local population density. We expected that a larger plant would have higher pollination efficiency because of the larger number of flowers to attract more pollinator insects (Klinkhamer et al. 1989). However, the percentages of immature seed fall and empty seeds were not affected by tree sizes but were instead influenced by local population density (Tab. 1). This indicates that the number of flowers at the local population level (several hundred square meter scale) was more important for pollination success than that at the individual level (several dozen square meter scale) for *A. mono*.

In contrast to *A. mono*, pollination efficiency of *Kalopanax pictus,* which is also a main component tree species in this forest, was not affected by local population density (Fujimori et al. 2006). We presume that such different responses between tree species reflect differences in pollinator foraging range and abundance. The most frequent flower visitors of *K. pictus* is the honeybee, which has a large foraging range (Fujimori et al. 2006); its foraging distance can range 1-10 km (Visscher and Seeley 1982; Sasaki 1999). Honeybees contributed to effective pollination in sparsely-distributed *Dinizia excelsa* trees (distance between trees was ca. 1500 m) in fragmented forests in Brazil (Dick et al. 2003). Honeybees in this forest may also contribute to effective pollination for *K. pictus*. The most frequent flower visitors of *A. mono* were small solitary bees, hoverflies, and flies. Such insects probably work as pollinators. They showed large variations in abundance and species composition with landscape change even over small distance (Steffan-Dewenter et al. 2002, Sueyoshi et al. 2003), indicating that small solitary bees and flies have a narrower foraging area and are sensitive to changes in stand conditions. We presume that pollination efficiency of *A. mono* varies with local population density due to changes in pollinator abundance and behavior in responding to the local flowering tree density.

We expected that local population density of the reciprocal sex morph rather than that of both types may have a stronger effect on pollination for *A.mono*. However, the difference between them was not so large; AIC of the multiple regression models constructed with DIS-RS had a slightly lower value than that with DIS-N. This is probably caused by mixed spatial distribution of both sex morphs. Local population density was similar in both the models, and DIS-RS had a high correlation with DIS-N (r=0.94). It will depend on the degree of deflection of spatial distribution of each sex morphs whether local density of the reciprocal sex morph is more important or not.

Heterodichogamous trait was important as an individual level factor. Trees of PG type had significant lower immature seed fall than PA (Tab. 1). This result suggests that the former had higher efficiency of pollen acceptance (bias to female function) than the latter. Therefore, heterodichogamy relates not only to the reciprocal pollination system but also to the difference of gender specialization linked with sex morph. Several reports said that heterodichogamy was one of evolutional pathway from monoecy to dioecy (Pendleton et al. 2000, Sato 2002). The bias of female function in PG in this study may reflect such evolutional background. In contrast to PG, PA, which has higher immature seed fall, may expend effort to pollen supply like *Juglans ailanthifolia* (Kimura et al. 2003).

Density dependent seed mortality

Density dependent mortality has been well reported at the post-dispersal seed stage and seedling stage for many species (Shibata and Nakashizuka 1995, Hille Ris Lambers and Clark 2003, Wright et al. 2005). Our results showed density dependent mortality is also occurring at the pre-dispersal seed stage (Tab. 1) similar to other tree species that have been studied recently: *Samanea saman* in a tropical forest (Cascante et al. 2002), and *K. pictus* (Fujimori et al. 2006) and *Magnolia obovata* in a temperate forest (Isagi et al. 2007). We think that density dependent mortality is also a common phenomenon in the fruiting process.

Steffan-Dewenter et al. (2001) pointed out that decreasing predation at a low population density site counterbalances the disadvantage of the low pollination success. Our study also detected a similar situation. As a consequence of seed set, population density did not affect soundness of seeds in the spatial scale of this study (Tab. 1). However, we have to be careful not to give a simple explanation that local population density does not influence reproduction, because different functional groups, namely pollinators and seed predators, counterbalance their effects with respect to local population density. Unfortunately, we do not currently know the critical spatial scale of the habitats for these functional groups in detail. This study shows that the effect of local population density on plant reproduction was very complicated. We have to examine further the counterbalance between pollination and seed mortality and this will be part of an investigation including a wider range of the population densities of host plants.

Factors affecting seed maturation

Contrary to our expectations, individual tree size did not have a clear relationship with either seed soundness or seed size (Tab. 1). On the other hand, growth of the seed was affected by local population density. These results suggest that seed development of this species is regulated more by pollination than by individual resource conditions. However, seed size tends to be rather smaller at high local population density sites (Tab. 1). If neighboring individuals at the high local population density site are closely related to each other, and pollinators remain within the site, inbreeding depression among neighboring trees will occur. We presume that downsizing of seed results from such inbreeding depression. This means that high local population density has a negative effect on the quality of pollination like biparental inbreeding (Ritland and Jain 1981). To examine this suggestion, it is important to investigate mechanisms of the reproductive consequence related to local population by revealing the actual pollen flow by genetic approaches as a part of future study.

Conclusion

This study showed that local population density and sex morph had effects on various stages of the fruiting process. Impacts operated both negatively and positively. This complicated interaction may lead to autonomous control of abundance of this species, as with *Magnolia obovata* (Isagi et al. 2007). However, the response of each species depends on the variety of pollinators and seed predators as mentioned above. The response of local population density may also change with annual fluctuation of seed production and insect population dynamics. To consider effects of human disturbance of forest on tree reproduction, it is

important to clarify plant-animal interaction in reproduction and in the long-term dynamics of target species.

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Table 1. Multiple regression analyses of factors affecting the fruiting process.

Demographic parameters	Distance from neighboring adults		Tree size		Sex morph		Regression model's	
	effect	Prob.	effect	Prob.	effect	Prob.	R^2	AIC
Seed mortality								
Pollination failure								
Immature seed fall	+	0.014	ns	---	PG<PA	0.005	0.41	151.8
Empty seeds	+	<.0001	ns	---	ns	---	0.26	437.4
Suffering predation	-	0.041	ns	---	ns	---	0.07	423.9
Decay	-	0.003	ns	---	ns	---	0.13	439.9
Seed maturation								
Soundness	ns	---	ns	---	ns	---	<.00	467.2
Seed kernel weight	(+)	0.071	ns	---			0.56	91.8

DIS-RS and DBH are shown as an indicator of distance from conspecific adults and tree size, respectively.

Symbols of effect are as follows; +: a positive effect, -: a negative effect, (+): marginally significant positive effect, ns: no significant effect.