

Geographical Variation in Seed Weight of *Plantago asiatica* L.*

Shigeru MARIKO¹⁾, Takashi NAKANO²⁾, Yukiko BEKKU²⁾
and Naoki KACHI³⁾**

¹⁾ *Sugadaira Montane Research Center, University of Tsukuba,
Sanada, Nagano 386-22, Japan*

²⁾ *Department of Biology, Faculty of Science, Tokyo Metropolitan University,
Hachiohji-shi, Tokyo 192-03, Japan*

³⁾ *Global Environment Research Division,
The National Institute for Environmental Studies, Tsukuba, Ibaraki 305, Japan*

(Accepted Sep. 7, 1995)

Synopsis

Plantago asiatica L. consists of two ecotypes: the northern ecotype and the southern one. The two ecotypes showed different patterns of correlations between seed weight and a geographical or thermal gradient. The mean seed weight of the southern-ecotype populations increased within 25-37°N of the latitude and decreased with the cumulative temperature; that of the northern-ecotype populations decreased within 38-45°N of the latitude and increased with the cumulative temperature. It seems that the preference of seed number is not so important to the southern-ecotype populations. Rather, the southern-ecotype populations at higher latitudes prefer to the strategy by which individuals produce heavier seeds at the expense of seed number, allowing germinated plants to grow and reproduce with advantage under a shorter growing season. As a result, the strategy seems to be reflected in the latitudinal pattern in the field. The northern ecotype is considered to have a propagation strategy by which small amounts of assimilated resources, restricted by the severe environments, are used to ensure seed number at the expense of seed weight. The results in the present study suggest the possibility of this idea.

Key words: Distribution, ecotype, geographical variation, *Plantago asiatica* L., seed weight

* Contributions from Sugadaira Montane Research Center, University of Tsukuba no. 146.

** Present address: Department of Biology, Faculty of Science, Tokyo Metropolitan University, Hachiohji-shi, Tokyo 192-03, Japan.

Introduction

Seed weight is an important factor influencing plant fitness (HARPER *et al.* 1970, JANZEN 1977) through dispersal and germination of seeds and emergence and survival of seedlings (BLACK 1957, HOW and VAN DE KERCKHOVE 1980, SCHAAL 1980, WEIS 1982, HENDRIX 1984, WINN 1985). As a consequence of seed-weight effects on seedling establishment, natural selection in determining seed weight may produce seed-weight variation within a widespread species which establishes over a wide environmental range along a geographical gradient (WINN 1988). There is a general trend of increasing seed weight and decreasing seed number per plant with shortening length of growing season, or with increasing latitude and altitude (JOHNSON and COOK 1968, MCWILLIAMS *et al.* 1968, KAWANO and MASUDA 1980, MARIKO *et al.* 1993, SAWADA *et al.* 1994).

Plantago asiatica L. is a perennial, rural weed distributed throughout Japan. This widespread species is divided into two ecotypes based on their phenological differences: summer green type (northern ecotype) and evergreen type (southern ecotype) (YAMANISHI and FUKUNAGA 1983). The geographical distribution of the two ecotypes in Japan is separated at about 37°N in latitude below 500 m altitude. Previous studies (YAMANISHI and FUKUNAGA 1983, SAWADA *et al.* 1994) show that the northern ecotype has a larger seed size than the southern ecotype, and that the seed weight of the southern-ecotype populations increases with increasing latitude. Such latitudinal variations are interpreted as an adaptation for seedlings to reach mature size more rapidly where the growing season is shorter. However, there has been little knowledge about how the between-ecotype and within-ecotype variations in seed weight exist in native habitats.

The main aim of the present study is to examine correlation between the mean seed weight of *P. asiatica* populations in native habitats and the latitude or cumulative temperature. The present study also examines a possibility of genetic variation in seed weight within the northern-ecotype populations.

Materials and Methods

Seed collection in native habitats

In order to study latitudinal and climatological variation among native seed populations, eighteen seed populations were collected at various latitudes below 500 m in altitude throughout Japan during September–November in 1992 (Table 1). These populations include six northern-ecotype populations, four populations on the distribution border between the northern and southern ecotypes, and eight southern-ecotype populations. Ripe seeds were sampled from ten well-established and matured individuals in each population. The seeds were air-dried in a desiccator at room temperature over a month. Thirty seeds were removed from all inflorescence of each individual and weighed as a lot. The seed weight of one population was represented as a mean seed weight of the ten individuals.

Table 1. Geographical and climatic information on seed collection sites.

	Ecotype ^{a)}	Latitude (N)	Altitude (m)	Cumulative temperature ^{b)} (degree day)	Growth period ^{c)} (day)
Shibetsu	N	44°11'	<150	1023	154
Sapporo	N	43°04'	< 50	1233	175
Obihiro	N	42°55'	< 50	1027	163
Kuroishi	N	40°38'	360	1197	173
Sakata	N	38°54'	30	1738	206
Sendai	N	38°16'	20	1655	205
Nagaoka	B	37°27'	< 50	2089	215
Shirakawa	B	37°12'	280	1742	207
Utsunomiya	B	36°33'	100	2049	223
Mito	B	36°23'	< 50	2046	229
Tsukuba	S	36°06'	30	2193	232
Urayasu	S	35°41'	1	2377	248
Kiyosumi	S	35°07'	70	2459	268
Nara	S	34°42'	100	2369	237
Fukuoka	S	33°52'	< 50	2608	260
Kagoshima	S	31°34'	< 50	2972	285
Okinawa	S	26°11'	< 50	4510	365
Yonaguni	S	24°28'	< 50	4916	365

^{a)} Ecotypic distinction: N, northern ecotype; S, southern ecotype; B, populations on the distribution boundary between the two ecotypes.

^{b)} Cumulative temperature was calculated by summing daily mean air temperatures above 10°C over a year.

^{c)} Growth period is a sum of days when daily mean air temperature is above 10°C.

Additional seed populations from various latitudes (25 populations) and altitude (23 populations) were collected for examining genetic variation among northern-ecotype populations. In this case, one population refers to one lot of ripe seeds collected from five individuals. The geographical range of collection sites was between 37°50' and 44°11'N in latitude and between 2 and 2,350 m in altitude.

Calculation of cumulative temperature

The cumulative temperature used as a climatological index at each collection site was calculated as follows. A standard climatological station was chosen for each site (Table 1) (JAPANESE METEOROLOGICAL AGENCY 1981, 1982). Annual changes in the daily mean air temperatures at the standard climatological station were estimated by interpolating the monthly normals of mean air temperature using a harmonic analysis, developed by the Japan Meteorological Agency. The estimated annual change was converted into the annual changes at each site, using the rates of temperature changes along an altitudinal or latitudinal gradient. The annual mean rate of temperature change with altitude in each collection site

was estimated to be within -0.57 to -0.65°C per 100 m (FUJIMURA 1971, JAPANESE METEOROLOGICAL AGENCY 1981, 1982, 1984, MASUZAWA *et al.* 1988). The latitudinal change was estimated at -0.97°C per 100 km on the Pacific-Ocean side and at -0.81°C per 100 km on the Japan-Sea side (JAPANESE METEOROLOGICAL AGENCY 1981, 1982).

The cumulative temperature at each site was calculated by summing the estimated daily mean temperatures above 10°C . The latitudinal distribution border of *P. asiatica* ecotypes (about 37°N) corresponded to the cumulative temperature of about 1,900 degree day (Table 1).

Seed collection in a greenhouse

A possibility of genetic influence on the seed weight of northern ecotype populations was examined by correlating the seed weight of parent's seed populations collected at native habitats with that of their offspring's seed populations under controlled environments in a greenhouse. The forty-eight parent's populations were used for this experiment. These populations were divided into two groups at the altitudinal boundary of 500 m which was conveniently fixed in order to examine the altitudinal influence on seed weight. The parent's seed populations had the mean seed weight of 0.281 to 0.832 mg per seed. The eight parent's seeds were sown. The weight of these seeds was almost identical with the mean weight of their belonging seed population.

Seeds were germinated in pots (12 cm diameter, 8 cm high) with artificial soil composed of peat moss, vermiculite, perlite, fine gravel and akadamatsuchi (granulated loam) (2 : 2 : 1 : 1 : 2 v/v), in the middle of April 1992. Germinated plants were thinned three per pot at the beginning of May, and then were grown in a greenhouse in which temperature fluctuated following the outdoors and photoperiod was constant at 14 h with supplementary light (fluorescent tubes). Plants were irrigated every day, and fertilized every-two weeks with about 200 ml of Hyponex solution (Hyponex Japan, 1 g l^{-1} , N : P : K = 11.8 : 1 : 5.1).

Before flowering of emergent inflorescences began in the greenhouse, at least two inflorescences were enclosed in a long and slender paper bag to avoid cross-pollination. The selfed seeds were collected separately for each plant on late of October, when most plants had produced ripened inflorescences. The seeds were air-dried in a desiccator at room temperature for three weeks. The weight per 100 seeds was measured to determine the mean weight of seeds of each offspring plant.

Data analysis

Correlations between the mean seed weight and the latitude or climate and between the mean seed weights of parents and offspring were statistically analyzed using KENDALL's rank correlation (τ). Difference between the mean seed weights was analyzed using *t* test. Significance level was $P=0.05$.

Results

The southern and northern ecotypes showed different patterns of significant correlations between seed weight and a geographical or thermal gradient (Fig. 1). The mean seed weight of the southern-ecotype populations correlated positively with the latitude ($\tau = -0.643$, $P < 0.05$, sample size = 8) and negatively with the cumulative temperature ($\tau = 0.619$, $P < 0.05$, sample size = 7). On the other hand, the mean seed weight of the northern-ecotype populations correlated negatively with the latitude ($\tau = 0.511$, $P < 0.05$, sample size = 10) and positively with the cumulative temperature ($\tau = -0.527$, $P < 0.05$, sample size = 11).

No correlation between the mean seed weights of parents and offsprings was found within the northern-ecotype populations: $\tau = 0.115$, $P \geq 0.05$, sample size = 25 for populations below 500 m; $\tau = 0.047$, $P \geq 0.05$, sample size = 23 for populations above 500 m; $\tau = 0.158$, $P \geq 0.05$, sample size = 48 for all populations (Fig. 2). The offspring plants grown from the parent's seed of 0.3 to 0.8 mg (0.520 ± 0.122 SD mg per seed, CV = 23.5%) produced the mean seed weights with a narrower range of 0.4 to 0.6 mg (0.500 ± 0.056 SD mg per seed, CV = 11.3%). There was no difference between the parent and offspring's seed weights (t test: $\tau = 1.102$, $P \geq 0.05$, sample size = 47).

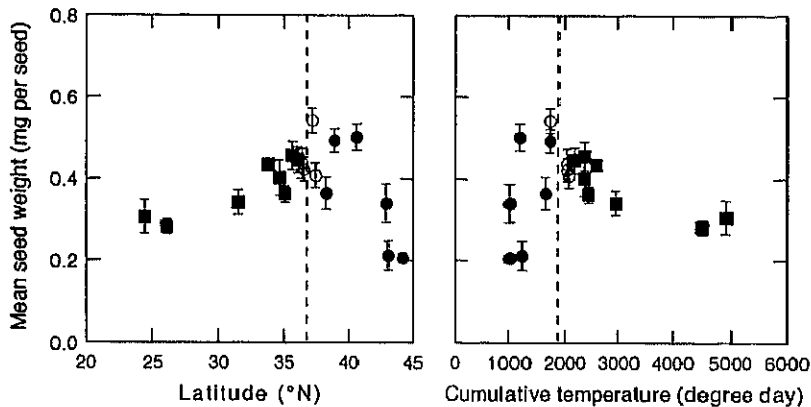


Fig. 1. Relationships between the mean seed weight of *P. asiatica* ecotypes and the latitude or the cumulative temperature. Symbols: (●) the northern-ecotype populations, (○) the populations on the distribution border, (■) the southern-ecotype populations. Dotted lines are the distribution border between the northern ecotype and the southern ecotype.

Discussion

Plantago asiatica propagates only by seeds which can be dispersed to some limited sites by animals and gravity. In such a plant, the number of produced seeds is important for the propagation strategy. However, the preference of seed number may be not so important to the southern ecotype, because the natural populations of the evergreen southern ecotype have a long flowering period, allowing them to produce seeds to a great extent even at higher latitudes within the distribution area (SCHAAL 1980, YAMANISHI and FUKUNAGA 1983). Rather, the southern-ecotype populations at higher latitudes may prefer to the strategy that individuals produce heavier seeds at the expense of seed number (SAWADA *et al.* 1994). This is the most possible idea to explain the reason why the differentiation for seed weight occurs within the southern-ecotype populations. The latitudinal cline observed in native habitats seems to show that the southern-ecotype populations at higher latitudes produced seeds with advantage under a shorter growing season (Fig. 1).

The propagation strategy to give priority to heavier seed weight may be unfavorable to the northern-ecotype populations. The northern-ecotype populations receive much smaller amounts of environmental resources and allocate much smaller amounts of photosynthate to seed production than the southern-ecotype populations. A favorable propagation strategy for the northern-ecotype populations seems to use the limited photosynthate to ensure seed number at the expense of seed weight (SAWADA *et al.* 1994). Therefore, in northern regions, there may be no priority of natural selection given to heavier seed weight. Direct evidence of this idea is not still presented. A possibility of non-genetic variation in seed weight, however, seems to be shown to some extent in the present study. First, the populations at higher latitudes produce smaller seeds (Fig. 1). The decreasing pattern may result from the

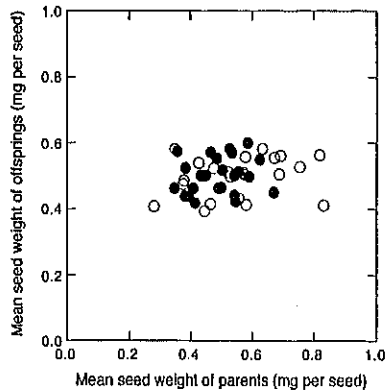


Fig. 2. Relationship between the mean seed weights of parents in native habitats and of offsprings grown in a greenhouse in the northern ecotype of *P. asiatica*. Symbols: 25 populations below 500 m (●) and 23 populations above 500 m (○) in altitude at various latitudes.

environmental variation (BLACK 1957, LALONDE and ROITBERG 1989). Second, there was no correlation between the mean seed weights of parents and offsprings (Fig. 2). The further study is required to examine heritability considering the variation within each northern-ecotype population.

Acknowledgments

We thank Prof. I. HAYASHI, University of Tsukuba, for his comments on this manuscript. We also thank many people for providing or collecting seeds : Y. YAMAMURA, T. OKUDA, M. MISHIO, M. YOKOTA, T. MARIKO, A. TAKEDA, T. TAKEDA, S. MIURA, H. TAKAHASHI, A. TAKEUCHI, K. NAKA, T. MASUMOTO and K. SHIBUYA.

References

- BLACK, J. N. (1957) The early vegetative growth of three strains of subterranean clover (*Trifolium subterraneum*) in relation to size of seed. *Aust. J. Agr. Res.* 8: 1-14.
- FUJIMURA, I. (1971) The climate and weather of Mt. Fuji, pp. 211-345. In "The Report of Comprehensive Scientific Investigation," Fuji Kyuko, Tokyo. (In Japanese with English summary).
- HARPER, J. L., P. H. LOVELL and K. G. MOORE (1970) The shapes and sizes of seeds. *Ann. Rev. Ecol. Syst.* 1: 327-356.
- HENDRIX, S. D. (1984) Variation in seed weight and its effects on germination in *Pastinaca sativa* L. (Umbelliferae). *Am. J. Bot.* 71: 795-802.
- HOWE, H. F. and G. A. VAN DE KERCKHOVE (1980) Nutmeg dispersal by tropical birds. *Science* 210: 925-927.
- JANZEN, D. H. (1977) Variation in seed size within a crop of Costa Rican *Mucuna andreana* (Leguminosae). *Am. J. Bot.* 64: 347-349.
- JAPANESE METEOROLOGICAL AGENCY (1981) Climatic Table of Japan. Part 1. Monthly Normals of Atmospheric Pressure, Temperature, Relative Humidity, Precipitation and Sunshine Duration, 1951-1980. The Japan Meteorological Agency, Tokyo. (In Japanese).
- JAPANESE METEOROLOGICAL AGENCY (1982) The Monthly Normals of Temperature and Precipitation at Climatological Stations in Japan (1951-1978). The Japan Meteorological Agency, Tokyo. (In Japanese).
- JAPANESE METEOROLOGICAL AGENCY (1984) Weather at the Summit of Mt. Fuji. No. 10. The Japan Meteorological Agency, Tokyo. (In Japanese).
- JOHNSON, M. P. and S. A. COOK (1968) 'Clutch size' in buttercups. *Am. Nat.* 102: 405-411.
- KAWANO, S. and J. MASUDA (1980) The productive and reproductive biology of flowering plants: VII. Resource allocation and reproductive capacity in wild populations of *Heloniopsis orientalis* (THUNB.) C. TANAKA (Liliaceae). *Oecologia* 45: 307-317.
- LALONDE, R. G. and B. D. ROITBERG (1989) Resource limitation and offspring size and number

- trade-offs in *Cirsium arvense* (Asteraceae). *Am. J. Bot.* **76** : 1107-1113.
- MARIKO S., H. KOIZUMI, J. SUZUKI and A. FURUKAWA (1993) Altitudinal variations in germination and growth responses of *Reynoutria japonica* populations on Mt. Fuji to a controlled thermal environment. *Ecol. Res.* **8** : 27-34.
- MASUZAWA, T., T. HAYASHI and S. NISHITANI (1988) Seasonal changes in the air temperature and the humidity over a four-year period at the timberline on Mt. Fuji. *Rep. Fac. Sci. Shizuoka Univ.* **23** : 65-72.
- MCWILLIAMS, E. L., R. Q. LANDERS and J. P. MAHLSTEDE (1968) Variation in seed weight and germination in populations of *Amaranthus retroflexus* L. *Ecology* **49** : 290-296.
- SAWADA, S., Y. NAKAJIMA, M. TSUKUDA, K. SASAKI, Y. HAZAMA, M. FUTATSUYA and A. WATANABE (1994) Ecotypic differentiation of dry matter production processes in relation to survivorship and reproductive potential in *Plantago asiatica* L. populations along climatic gradients. *Funct. Ecol.* **8** : 400-409.
- SCHAAL, B. A. (1980) Reproductive capacity and seed size in *Lupinus texensis*. *Am. J. Bot.* **67** : 703-709.
- WEIS, I. M. (1982) The effects of propagule size on germination and seedling growth in *Mirabilis hirsuta*. *Can. J. Bot.* **60** : 1868-1874.
- WINN, A. A. (1985) The effects of seed size and microsite on seedling emergence in *Prunella vulgaris*. *J. Ecol.* **73** : 831-840.
- WINN, A. A. (1988) Ecological and evolutionary consequences of seed size in *Prunella vulgaris*. *Ecology* **69** : 1537-1544.
- WULFF, R. D. (1986) Seed size variation in *Desmodium paniculatum* I. Factors affecting seed size. *J. Ecol.* **74** : 87-97
- YAMANISHI, H. and N. FUKUNAGA (1983) Ecotypic differentiation of *Plantago asiatica* L. in Japan Islands. *Jpn. J. Ecol.* **33** : 473-480. (In Japanese with English summary).