Evaluation of adaptation to myrmecochory in etaerio-bearing plants Duchesnea chrysantha and D. indica（Rosaceae）

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Shinya Ishikawa[1, 2] and Naohiro Naruhashi[1] : **Evaluation of adaptation to myrmecochory in etaerio-bearing plants *Duchesnea chrysantha* and *D. indica* (Rosaceae)**

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Abstract

Duchesnea (Rosaceae) has fruits (etaerios) like strawberry (*Fragaria*) and is so far thought to be a bird-disseminated plant. However, its achenes possess appendages that are morphologically like elaiosomes, suggesting that ants would disseminate those achenes and seeds. In 1998 and 1999, we tested whether achenes (seeds) of two closely-related species *D. chrysantha* and *D. indica*, growing in Kurehayama Park and the surroundings, Toyama City, central Japan, would be removed and dispersed or not removed by ants. Four ant species (*Lasius niger*, *Paratrechina flavipes*, *Pristomyrmex pungens* and *Tetramorium caespitum*) were observed to carry the achenes to their nests. Ants frequently removed achenes of *D. chrysantha* but did not remove those of *D. indica*. Viable seeds of *D. chrysantha* were found at a depth from 0 to 40 cm in the soils, while those of *D. indica* were found only at the top layer (0 to 10 cm). These results suggest that the achenes of *D. chrysantha* may be effectively dispersed by ants rather than those of *D. indica*. We consider that *D. chrysantha* is amphichorous, i.e., etaerios are eaten and achenes are disseminated by birds and in the case of fruits that have not been eaten by birds, achenes drop off from the receptacle and are removed and dispersed by ants.

Key words : amphichory, ant, *Duchesnea*, myrmecochory, seed dispersal.

Duchesnea (Rosaceae) consists of perennial herbs that show two types of reproduction, i.e. sexual reproduction by seeds as a result of blooming at spring and fruiting in early summer, and asexual reproduction by juvenile plants rooting from nodes of runners. The genus has two species, *D. chrysantha* (Zoll. et Moritzi) Miq., 2n=14 (diploid) and *D. indica* (Andrews) Focke, 2n=84 (dodecaploid) in the world (Hara and Kurosawa 1959 ; Robertson 1974).

Both species at a glance are morphologically very similar except for body size. For this reason, Kalkman (1968) made taxonomically no difference between them and Kitamura and Murata (1961) treated them as different at variety level. On the contrary, many taxonomists treated them as two distinct species (Ohwi 1965 ; Okuyama 1977 ; Momiyama 1982 ; Kitagawa 1983 ; Yü and Kuan 1985 ; Naruhashi 2001).

We have done detailed studies from points of view of morphology, cytology and ecology for resolving the relationship of the two species (Sugimoto and Naruhashi 1981, 1982 ; Kume et al. 1987 ; Sugimoto et al. 1991 ; Naruhashi and Ishizu 1992 ; Naruhashi and Sugimoto 1996).

Fruits of *Duchesnea* are a false fruit, the so-called "etaerio" which consists of an enlarging receptacle and many real fruits, each containing one seed. One of the disseminules of *Duchesnea*, the etaerio, is eaten by birds, the other disseminule, the achene, is the real fruit situated on the enlarging receptacle. The former settles in cluster, while the latter moves separately.

Duchesnea is distributed throughout Asia from Afghanistan to Japan, and its dispersal takes place by birds because plants occur in solitary islands (Shimizu 1967 ; Hatusima 1991).

In the genus *Fragaria* that is closely related to *Duchesnea*, no achenes drop down from the enlarging receptacle because they are firmly sunk in the receptacle. Achenes of *Duchesnea*, however, protrude considerably from the receptacle and drop down easily even when touched lightly.

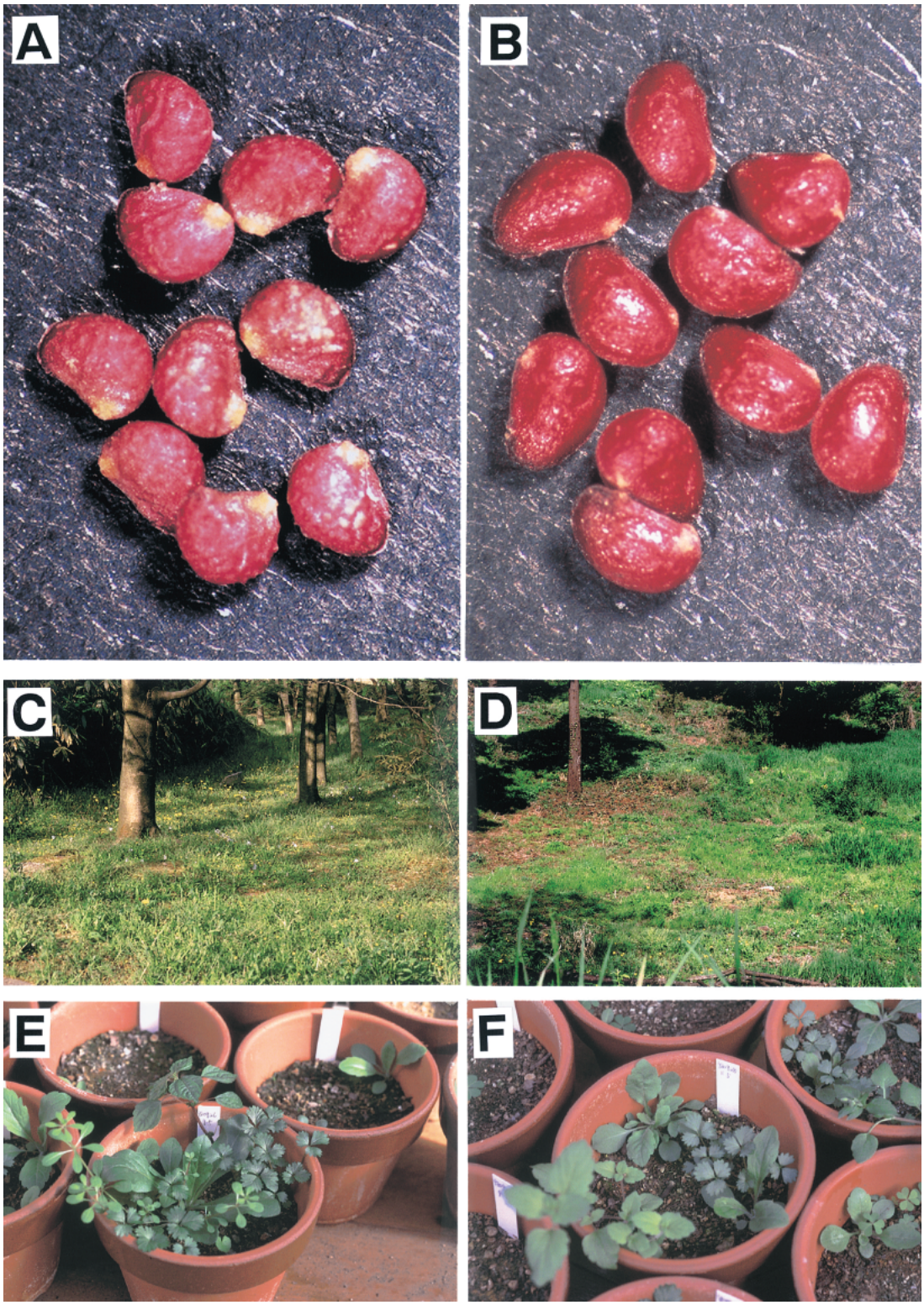

Fig. 1. Achenes of *Duchesnea chrysantha* (A) and *D. indica* (B), experimental site of *D. chrysantha* (C) and of *D. indica* (D), and pots of germination experiment (E, F).

While, *Potentilla*, which is also closely related to *Duchesnea*, has no enlarging receptacle and its achenes drop down from the receptacle and some species of *Potentilla* were reported to be myrmecochorous (Nakanishi 1993, 1999), it was therefore expected that *Duchesnea* plants also could possibly be myrmecochorous.

Duchesnea chrysantha prefers sunny places such as open sites in paddy fields and those around houses, etc. and grows like a mat with synchronous blooming from late April to early May. Its fruiting period is late May to middle June. The achenes are red, with a rugose-tubercled surface, and yellow appendages on both sides of the hilum (Fig. 1 A). These appendages become inconspicuous after elapsing of a long time and after drying (Ishikawa 1994; Nakayama et al. 2000).

Duchesnea indica is found in places with low light intensity such as forest margins, woodlands, etc. It grows sparsely and opens flowers, one after another, from early May, without synchronous blooming as observed in *D. chrysantha* (Naruhashi and Sugimoto 1996). The fruiting period is from early June. Achenes are glossy smooth, they possess very small appendages or are sometimes obscure (Fig. 1 B). Supposing that these appendages are elaiosomes for attracting ants, ants might carry the achenes deep into their nests. If this is the case, there would be viable achenes deep in the soil.

To make clear myrmecochory of *Duchesnea*, we made preliminary experiments as to whether ants carry achenes or not. We also made two experiments on how do ants carry out achenes, and whether viable achenes do exist deep in the soil or not.

Materials and methods

Plants growing in Kureha Park and a neighboring open place (N 36°42′, E 137°04′) in Toyama City were used as materials (Figs. 1 C and D). Population of *D. chrysantha* consists of several patches and a path between them. In the park tall trees of *Robinia pseudoacacia* L., *Aesculus turbinata* Blume, *Quercus myrsinifolia* Blume and *Zelkova serrata* (Thunb.) Makino were cultivated and *D. chrysantha* grows around the trees. A list of the herbaceous plants growing with or near by *D. chrysantha* is shown in Table 1. A few mowings a year are carried out in the place.

The habitat of *D. indica* is in the neighborhood of the park. *Cryptomeria japonica* (L.f.) D. Don, *Robinia pseudoacacia* L. and *Ailanthus altissima* (Mill.) Swingle grow outside of the area. Inside, the area is open and becomes hollow, in which *D. indica* grows with somewhat tall grasses. Herbaceous plants growing in the area are listed in Table 2.

Matured achenes for experiment of *D. chrysantha* were collected at Kureha Park and those of *D. indica* at the neighborhood of Kureha Park. The mean fresh weight of an achene of *D. chrythanta* was 0.29 mg (about 3,000 in total) and of *D. indica* 0.46 mg (about 2,000 in total), respectively.

As preliminary experiments, several tens of achenes of *D. chrysantha* or of *D. indica* were put on a paper dish (5 cm × 5 cm) and left in each population. Since ant workers carried the achenes, we caught the ants for identification and used 10 individuals per species for measurment of body size. No other insects in the experiment carried out achenes. The days during which ants were caught were June 10, 1998 and June 16, 1999.

Experiment 1. How do ants carry achenes?

A) Which achenes of *D. chrythanta* or those of *D. indica* do ants prefer?

A sample is 20 achenes on a paper dish (5 cm × 5 cm). One lot of ten samples of *D. chrysantha* and ten samples of *D. indica* were left in the population of *D. chrysantha* and another lot was put in the population of *D. indica*. This experiment was carried out on 2nd and 4th of June, 1998.

Ten samples of *D. chrysantha* were left in the population in *D. chrysantha* and ten samples of *D. indica* in the population in *D. indica*. This experiment was carried out on 14th and 15th of June, 1999. These experiments were started at 10 o'clock in the morning and the numbers of achenes on the paper dishes were counted at 13, 16 and 19 o'clock by a 3-hour interval.

B) Is there a difference between achenes with or

Table 1. A list of herbaceous plants in or in the neighborhood of *Duchesnea chrysantha* population

Achyranthes bidentata Blume var. *tomentosa*（Honda）H.Hara
Ajuga decumbens Thunb.
Antenoron filiforme（Thunb.）Roberty et Vautier
Artemisia princeps Pamp.
Aster ageratoides Turcz. ssp. *ovatus*（Franch. et Sav.）Kitam.
Bothriospermum tenellum（Hornem.）Fisch. et C.A.Mey.
Capsella bursa-pastoris Medicus
Cardamine flexuosa With.
Carex sp.
Cayratia japonica（Thunb.）Gagnep.
Chamaele decumbens（Thunb.）Makino
Chrysosplenium japonicum（Maxim.）Makino
Clinopodium gracile（Benth.）Kuntze
Commelina communis L.
Duchesnea chrysantha（Zoll. et Moritzi）Miq.
Erigeron annuus（L.）Pers.
Erigeron canadensis L.
Erigeron philadelphicus L.
Galium spurium L. var. ***echinospermum***（Wallr.）Hayek
Geranium thunbergii Siebold et Zucc.
Geum japonicum Thunb.
Glechoma hederacea L. ssp. *grandis*（A.Gray）H.Hara
Houttuynia cordata Thunb.
Hydrocotyle maritima Honda
Hydrocotyle ramiflora Maxim.
Lapsana apogonoides Maxim.
Lysimachia japonica Thunb.
Medicago lupulina L.
Myosoton aquaticum（L.）Moench.
Ophiopogon japonicus（L.f.）Ker Gawl.
Oplismenus undulatifolius（L.Ard.）Roem. et Schult.
Oxalis corniculata L.
Paederia scandens（Lour.）Merr.
Pilea hamaoi Makino
Plantago asiatica L.
Poa annua L.
Poa sphondylodes Trin.
Ranunculus japonicus Thunb.
Reynoutria japonica Houtt.
Rorippa indica（L.）Hiern
Rorippa islandica（Oeder）Borbás
Rumex acetosa L.
Rumex japonicus Houtt.
Sagina japonica（Sw.）Ohwi
Sedum bulbiferum Makino
Sonchus oleraceus L.
Stellaria alsina Grimm var. ***undulata***（Thunb.）Ohwi
Stellaria media（L.）Vill.
Stellaria neglecta Weihe
Taraxacum officinale Weber
Trifolium repens L.
Veronica arvensis L.
Veronica persica Poir.
Vicia angustifolia L.
Vicia hirsuta（L.）Gray
Viola verecunda A.Gray

Plants germinated from seeds in soil（Exp. 2, 1998 and 1999）are shown in bold letters.

Table 2. A list of herbaceous plants in or in the neighborhood of *Duchesnea indica* population

Achyranthes bidentata Blume var. *tomentosa* (Honda) H.Hara
Agropyron tsukushiense (Honda) Ohwi var. *transiens* (Hack.) Ohwi
Ampelopsis brevipedunculata (Maxim.) Trautv. var. *heterophylla* (Thunb.) H.Hara
Amphicarpaea bracteata (L.) Fernald ssp. *edgeworthii* (Benth.) H.Ohashi var. *japonica* (Oliv.) H.Ohashi
Antenoron filiforme (Thunb.) Roberty et Vautier
Artemisia princeps Pamp.
Aster ageratoides Turcz. ssp. *ovatus* (Franch. et Sav.) Kitam.
Cardamine flexuosa With.
Cayratia japonica (Thunb.) Gagnep.
Cerastium holosteoides Fries var. ***angustifolium*** (Franch.) M.Mizush.
Chamaele decumbens (Thunb.) Makino
Cryptotaenia japonica Hassk.
Duchesnea indica (Andrews) Focke
Erigeron philadelphicus L.
Galium spurium L. var. *echinospermum* (Wallr.) Hayek
Geranium thunbergii Siebold et Zucc.
Geum japonicum Thunb.
Glechoma hederacea L. ssp. *grandis* (A.Gray) H.Hara
Houttuynia cordata Thunb.
Humulus japonicus Siebold et Zucc.
Lapsana humilis (Thunb.) Makino
Lycopus lucidus Turcz.
Mentha arvensis L. var. *piperascens* Malinv.
Miscanthus sinensis Andersson
Myosoton aquaticum (L.) Moench.
Osmorhiza aristata (Thunb.) Rydb.
Oxalis corniculata L.
Persicaria longiseta (Bruyn) Kitag.
Persicaria thunbergii (Siebold et Zucc.) H.Gross
Pueraria lobata (Willd.) Ohwi
Rumex acetosa L.
Stellaria media (L.) Vill.
Taraxacum officinale Weber
Torilis scabra (Thunb.) DC.
Trifolium repens L.
Veronica arvensis L.
Veronica persica Poir.
Youngia japonica (L.) DC.
Zingiber mioga (Thunb.) Roscoe

Plants germinated from seeds in soil (Exp. 2, 1999) are shown in bold letters.

without appendages for removal by ants?

Appendages of achenes were removed by sandpaper. These ten achens without appendages as treated sample and an untreated sample (with appendages) were left near the nestholes of ants (*Tetramorium caespitum*) after which the number of remaining achenes was counted. These experiments were carried out both in a population of *D. chrysantha* and of *D. indica* for 1 hour, from 10 o'clock of 31st of May, 1998.

Experiment 2. Are there any viable achenes buried in the soil?

Borings were carried out in 12 sites in total in the growing area of *D. chrysantha* in Kureha Park on 18th, 24th and 26th of September, 1998. Furthermore, in 1999 borings of 12 sites of *D. chrysantha* were carried out on 18th and 19th of September and at 12 sites of *D. indica* in the growing area of *D. indica* in the neighborhood of the park on 4th, 5th, 8th and 9th of October.

We obtained a column of soil (1 cm in diameter × 50 cm in length) by using the boring machine. The column of soil obtained was cut each 10 cm in length and then put into polyethylene bags with labels in the field. A column of soil made 5

samples of soil according to depth (0–10, 10–20, 20–30, 30–40 and 40–50 cm). These soil samples were put uniformly on heat-treated soil (using oven by 140℃, for 3 hrs) in #5 sized-flower pots (15.5 cm in diameter, 12.5 cm in height) in laboratory and were labeled. The flowerpots were put into a green house and the surface of the soil was kept moist by tender irrigation. After germination of some plants the pots were kept a long time for identification (Figs. 1 E and F).

Results

Ants that removed achenes belonged to four species and their mean body sizes were as followed :

Lasius niger (L.) (mean±S.D., 3.71±0.21 mm) ; *Paratrechina flavipes* (Fr. Smith) (2.44±0.28) ; *Pristomyrmex pungens* Mayr (3.11±0.24) ; *Tetramorium caespitum* (L.) (3.30±0.28).

Minimum size was 2.0 mm in *Pa. flavipes* and maximum size 4.0 mm in *L. niger*. These ants are very common in Japan (Azuma 1977). *Paratrechina flavipes* was observed only in the population of *D. indica*, while the other three species were found in both populations.

We observed that *L. niger* workers began to carry an achen as soon as they found it accidentally. And then other individuals, which we thought to belong to the same colony, appeared and took away achenes. *Pristomyrmex pungens* examined an achene, spending time and then carried it when he found it. *Tetramorium caespitum* acted as *Pr. pungens* did. We also observed there were individuals, who carried an achene and other individuals that never carried it after their failures to bite off appendages. *Paratrechina flavipes* took more time to remove achenes than *Pr. pungens* and *L. niger* did. As a result of our observation, *T. caespitum* was strongly interested in achenes and removed them. All four ant species were interested in achenes of *D. chrysantha*, while they were hardly interested in those of *D. indica* except for *L. niger*.

Besids the four ants, *Formica japonica* Motschulsky (mean size±S.D., 6.28±0.41 mm ; min. -max., 5.8–6.9 ; n=10) and *Camponotus japonicus* Mayr (size, 11.1 mm ; n=1) were found in the experimental area. These two ants were not interested in achenes of *Duchesnea*.

How fast do ants carry achenes?

In Exp. 1 (A) , removed achenes of *D. chrysantha* and *D. indica* in the same plant population of same or different plant species are shown in Fig. 2. Ants in both plant populations remarkably removed achenes of *D. chrysantha* and this was more conspicuous in *D. indica* population. While achenes of *D. indica* were merely removed in both populations and there was no difference between populations. The removal frequency of the two species achenes in the same population showed some difference between 1998 and 1999, i.e., the value of 1999 was less than that of 1998. The reason is not obvious, althought it might be related to weather, especially temperature. In this experiment achenes were removed remarkably in the period from 10 to 13 o'clock than in other times.

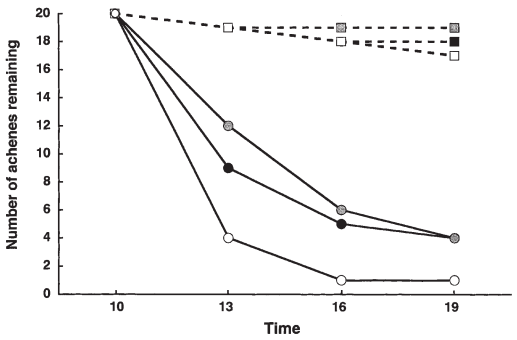

Fig. 2. Removal of *Duchesnea chrysantha* (Dc) and *D. indica* (Di) achenes by ants in own and the other populations (Means of 10 achenes were shown as circles and squares). Experiment was started at 10 o'clock in the morning. The number of achenes remained was counted at 13, 16 and 19 o'clock, respectively. Circles, Dc ; squares, Di. Black circles and squares, in own population in 1998 ; gray circles and squares, in own population in 1999 ; white circles and squares, in other population in 1998.

In Exp. 1 (B), the difference of removed achenes by *T. caespitum* between the groups "achenes with appendages" and "achenes without appendages" in one hour is shown in Fig. 3. The most removed achenes were those of *D. chrysantha* with appendage, which were completely removed during 30 minutes. Achenes without appendages of *D. chrysantha* were merely removed and showed remarkable difference compared

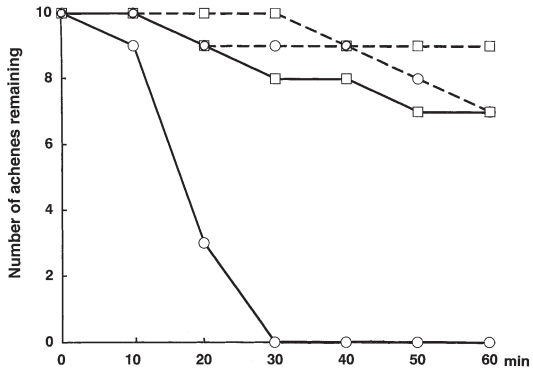

Fig. 3. Removal of *Duchesnea chrysantha* (Dc) and *D. indica* (Di) achenes by ants (*Tetramorium caespitum*) with or without appendages (Mean of 10 achens were shown as circles and squares). Transversal axis is minute. Circles, Dc; squares, Di. Solid line, with appendage; dashed line, without appendage.

with achenes with appendages. Achenes with appendages of *D. indica* were merely removed and much less than achenes of *D. chrysantha*. Achenes without appendages of *D. indica* were hardly removed.

Experiment 2. Are there any viable achenes buried in the soil?

The result of germination test from soil borings is shown in Fig. 4.

From the soil obtained from population of *D. chrysantha*, the result of 1998 is shown in column A of Fig. 4. Plants germinated are ten species in total as follows:

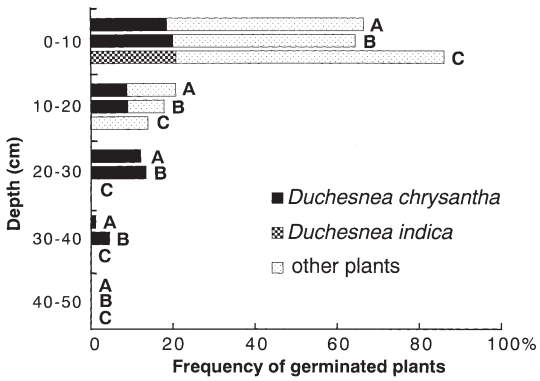

Fig. 4. Frequency of germinated plants from buried "seeds" in each depth of boring core in *Duchesnea* population (A, 1998 in *D. chrysantha* population; B, 1999 in *D. chrysantha* population; C, 1999 in *D. indica* population).

Cardamine flexuosa With; *Chrysosplenium japonicum* (Maxim.) Makino; *Galium spurium* L. var. *echinospermum* (Wallr.) Hayek; *Oxalis corniculata* L.; *Pilea hamaoi* Makino; *Sagina japonica* (Sw.) Ohwi; *Stellaria alsina* Grimm var. *undulata* (Thunb.) Ohwi; *S. media* (L.) Vill.; *Veronica persica* Poir.; *D. chrysantha*.

All of them growing in the experimental area (Table 1). Their achenes were buried at a depth of 0–10 cm and both the number of species as well as the number of individuals decreased sharply with soil depth. From soil layer at 10–20 cm depth, six species; *Ca. flexuosa*, *Ch. japonicum*, *O. corniculata*, *Sa. japonica*, *V. persica* and *D. chrysantha* germinated. From the layers at 20–30 cm and 30–40 cm depth, only *D. chrysantha* germinated. No plants germinated from the 40–50 cm layer. In total 92 plants geminated (61 plants at a depth of 0–10 cm; 19 at 10–20 cm; 11 at 20–30 cm; 1 at 30–40 cm) and of these the plants from 0–10 cm layer were 67.7%.

Similarly, the result of the germination test in 1999 from the soil obtained at population of *D. chrysantha* is shown in column B of Fig. 4. The plants germinated belonged to six species, such as *Artemisia princeps* Pamp., *G. spurium*, *O. corniculata*, *Sa. japonica*, *V. persica*, and *D. chrysantha*. From the 10–20 cm depth soil layer, three species, *O. corniculata*, *V. persica*, and *D. chrysantha*, and from those of 20–30 cm and 30–40 cm layers, only *D. chrysantha* germinated, respectively. No plants germinated from the 40–50 cm layer. In total only 45 plants germinated (0–10 cm, 29 plants; 10–20 cm: 8; 20–30 cm, 6; 30–40 cm, 2) and of these the plants from 0–10 cm layer were 64.4%.

Ninety-two plants in total germinated in 1998. In 1999, however, only 45 plants emerged in total representing about half of the amount registered in 1998. And plants germinated in 1998 were hardly diffferent from those of 1999. The above 1998 and 1999 germination results show that plants geminated from the 0–10 cm layer constituted by large the overwhelming majority.

From the soil at population of *D. indica* the result of 1999 germination test is shown in column C of Fig. 4. The plants geminated belonged to seven species: *Ca. flexuosa*, *O. corniculata*,

Cerastium holosteoides Fries var. *angustifolium* (Franch.) M.Mizush., *St. media*, *Trifolium repens* L., *V. persica*, *D. indica*, which were plants growing in the experimental area (Table 2). All plants germinated from the 0–10 cm layer. *Oxalis corniculata* germinated from the 10–20 cm layer, however no plants germinated from lower depths. *Duchesnea indica* germinated only from the 0–10 cm layer. In total 29 plants germinated (25 plants at a depth of 0–10 cm ; 4 at 10–20 cm ; 0 at 20–30 cm ; 0 at 30–40 cm) and of these plants from the 0–10 cm layer were 86.2%.

Discussion

Ants preferred remarkably to carry *D. chrysantha* achenes with appendages rather than those without appendages. These appendages are morphologically similar to those of myrmecochorous plants (Nakanishi 1988), so that they can be regarded as elaiosomes for food resources for ants.

The reason why achenes of *D. chrysantha* are preferred to those of *D. indica* is explained by the existence of appendages in the former. Also the difference of achene surface texture plays a role, i.e., rugose-turbecled in *D. chrysantha*, while surface of *D. indica* achenes is smooth. Nakanishi (1994) reported that the smooth seeds in *Corydalis* were unadaptive to myrmecochory. *Duchesnea indica* is not accounted for its achenes in terms of mymechochory, because 1) achenes have smooth surface and small or obscure appendages, 2) achenes were hardly ever being carried (Figs. 2 and 3), and no viable achenes were found below 10 cm depth in the soil (Fig. 4).

Duchesnea chrysantha grows like mat with synchronous blooming and fruiting and has high production of achenes per unit area. Therefore myrmechocory may be very convenient for dispersion of its disseminules. Conversely *D. indica* grows sparsely and opens flowers one after another without synchronous blooming (Naruhashi and Sgimoto 1996) and their fruits are observed even in autumn. In this plant, 1) fruit maturity is not strongly synchrous and 2) long distance among fruits with long runners and long internodes, show lower density for concentration of fruits than that of *D. chrysantha*. This suggests that *D. indica* may be not strongly adaptive for myrmecochory, i.e., the plants might not need short dispersal by ants. On the basis of these results of the two experiments, *D. chrysanatha* is thought to be a myrmecochous plant, while *D. indica* is considered as less-adaptive to myrmecochory. This is the first report stating that *D. chrysantha* is myrmecochorous.

In Kureha Hill belonging to our experimental area, 30 species of ants were reported (Nakagawa et al. 1994). Only four of 30 species are recognized as seed-dispersal ants in the present experiment and they are contained in the lists of ants as seed-dispersal ants (Tanaka 1926 ; Hiura 1978 ; Kurosaki 1979 ; Nakanishi 1988, 1993, 1999).

Diplochory in *Viola* (Violaceae) by Beattie and Lyons (1975) and Ohkawara and Higashi (1994) and *Corydalis* (Papaveraceae) by Nakanishi (1994) are reported, i.e. seeds disperse by ballistic way and thereafter by ants. Nakanishi (1988) showed another diplochory where seeds contained in the faeces of mammals or birds were frequently observed being carried away by ants. In the case of *D. chrysantha* it may be not "real diplochory", but is accounted for dispersal in terms of amphichory (dispersal of two kinds). Achenes of *D. chrysantha* are located on the enlarging receptacle and they are totally made into a red etaerio. The etaerios are attractive to birds. When etaerios are not eaten by birds, their achenes drop off from the receptacle. Then ants may carry the achenes to their nests. Thus, we consider that *D. chrysantha* may be amphichorous, i.e., seed dispersal depends on frugivorous birds at first, even when dispersal occurring at low probability, and then secondary depends on ants to compensate the first alternative fail.

That "seeds" of *Duchesnea* exist in the soil is already known ; (Tsuji et al. 1983 ; Minaki and Tsuji 1996) . Kasahara (1977) reported that the seeds of *D. chrysantha* from seeds from a habitation site in late Yayoi Era (1,800 years ago) could germinate.

Achenes of *D. chrysantha* are removed by ants and buried in the soil forming a "seed bank" and they may germinate later in favourable conditions. This is a temporal strategy for dispersal of disseminules of *D. chrysantha* and the plant is thought to be a weed occurring in paddy fields,

plowed fields, parks and open spaces around houses.

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石川真也[1,2]・鳴橋直弘[1]：イチゴ状果を産するヘビイチゴとヤブヘビイチゴ（バラ科）におけるアリ散布への適応の評価

富山市呉羽山公園と隣接する空地に於いて，1998 年と 1999 年にヘビイチゴとヤブヘビイチゴの痩果を用いアリが運ぶかどうかの実験を行ったところ，4 種のアリ（トビイロケアリ，アメイロアリ，アミメアリ，トビイロシワアリ）が運搬した。最もよく運搬したトビイロシワアリを使用し，ヘビイチゴ群落内とヤブヘビイチゴ群落内でそれぞれどちらの痩果がよく運搬されるのかを調べたところ，ヘビイチゴの痩果が顕著に運搬された。一方，ヤブヘビイチゴはほとんど運搬されなかった。また，痩果の付属体の有無でアリの運搬の差を調べたところ，付属体の未除去のヘビイチゴが非常によく運搬された。また，これら付属体は種子の付着部の発達したもので，多くの被子植物のエライオソームに類似していた（中西 1988 の図参照）。秋に上記の場所でボーリングを行い，50 cm の土のコアーを取り，10 cm 毎のサンプルに分け，それぞれのサンプルからの発芽植物を調べた。その結果，10 cm 以上の深さから出土したヘビイチゴ種子の発芽を確認した。

以上の結果から，ヘビイチゴの痩果はアリ散布であり，この植物は鳥散布に加えて，アリ散布も行う二又散布であると考えられた。

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