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Within-Inflorescence Variation in Seed Set of White Clover (*Trifolium repens* L.) and Its Consequence for Seed Size Variation

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Synopsis

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Each inflorescence of white clover bears many florets. They set seeds at highly varying rates. We examined the extent of this within-inflorescence variation for a field population and its consequence for seed size variation. A considerable variation in seed set at floret level was observed in the population. This variation was partially attributable to floret position effect, upper florets on inflorescence having significantly less seed set and smaller seed size than lower florets. Seed set was highly variable even among florets at near site on the same inflorescence. Nevertheless, white clover was likely to produce almost the same sized-seeds regardless of seed number per floret. This trend was stronger in lower florets than in upper florets. It is suggested that there is the possibility of selective investment of resource in the upper part of inflorescence.

Key words : Seed set, Seed size, Within-inflorescence variation, *Trifolium repens*

Introduction

Seed set and seed size are basic traits which affect reproductive ecology of plants strongly. White clover has flower head-like inflorescence, each of which bears many florets, usually 20 to 150. Each floret involves about six ovules[3]. However, only a part of those develops into seeds, so that seed set rate (i.e., the ratio of seed number to ovule number) is largely less than 1.0[4,6]. Even within the same inflorescence, seed set is usually variable among florets[3]. Some florets develop no seeds, and others develop more than four seeds. Seed set of white clover is characterized as being low and variable. Why the seed set is variable, and what consequences of this phenomenon have for seed size variation? These questions are of great interest from the viewpoint of reproductive ecology.

The variation in seed set is partially attributable to floret position effect. MARSHALL *et al.*[3] observed from the glasshouse experiment that upper florets forming on inflorescence set lower number of seeds than lower florets. They suggested that the reason for this is the resource limitation. Flowering is later in upper florets than in lower florets. Resultantly, upper florets usually use the limited amount of resource for the development of seeds, whereas lower florets have the advantages in utilizing resource and developing seeds. This explanation leads to the idea that upper florets can produce smaller seeds than lower florets. Unfortunately, few data on the seed size variation have been presented yet, especially for field populations.

Furthermore, seed set rate seems to be highly variable even among florets at near sites on the same inflorescence (SAWADA, personal observation). Does this phenomenon occur frequently in fields? This paper examined the seed set and seed size variations within inflorescence of a field population. Following questions were addressed : (1) To what extent are the variations observed in the population? (2) How large is the magnitude of the differences in average seed set and seed size between the upper and lower florets? (3) What consequence does the seed set variation have for the seed size variation?

Materials and Methods

1. Materials

The study population is from a *Miscanthus sinensis*-dominated grassland, which is located in Tohoku Agricultural Experimental Station (Morioka, Iwate Prefecture ; 170 m in altitude). The grassland has been cut once to twice per year. A collection of inflorescence of white clover was performed on 19 July 1994. The inflorescences, whose color changed to dark brown and petioles lightly brown, were randomly sampled and after that, they were air-dried and stored in the laboratory.

2. Measurement

A preliminary measurement was made in order to know whether floret position effects on seed set and seed size occur or not. Ten inflorescences, which randomly selected, were measured for their pod number, seed number per pod and seed size variation. The procedure was as follows : First, for each inflorescence, all pods were individually examined with tweezers carefully and counted for seed number. Here, a seed refers to a visibly healthy seed. An immature seed is not included. Second, a mass of seeds (pooled over four successive pods) was weighed at 0.1 mg level. Then, average seed weight (i.e., average seed size) was obtained by dividing the mass weight by the number of seeds weighed. The preliminary measurement clearly showed that seed set and seed size were highly variable among pods within the same inflorescence, and that upper one-thirds of the total pods generally contained fewer and smaller seeds than lower pods. Thus, we classified pods of each inflorescence into two positions, the upper and lower, in the subsequent measurement. The upper position was defined as pods at upper one-third of an inflorescence, and the lower position as those at lower two-thirds.

Fifty inflorescences, which were randomly selected, were measured for their pod number, seed number per pod and seed size variation in order to examine the relationship between seed set and seed number. The procedure was as follows : First, for each inflorescence, all pods were classified into the upper and lower positions. Second, they were individually examined with tweezers carefully and counted for seed number. For each position of pod, further classification of pod was made according to seed number. That is, further seven classes (0-seeded, 1-seeded, 2-seeded, 3-seeded, 4-seeded, 5-seeded and 6-seeded pods) were added. Third, for each class of pod, a mass of seeds (eight to ten seeds in principle ; pooled over two to eight pods) was weighed at 0.1 mg level. Then, average seed weight was obtained by dividing the mass weight by the number of seeds weighed. For 1-seeded class, the number of seeds for weighing was often fewer than eight due to only one seed per pod.

3. Statistical analysis

Means, standard errors and ranges for various reproductive characters were calculated based on the measurement of the fifty inflorescences. Frequencies of the seven classes of pod according to seed number were also obtained based on this data. F-test was performed to test the significance of the difference in seed set and seed size between the upper and lower positions. The test indicated significant differences, so that the relationship between seed set and seed number was analyzed each for the upper and lower position. Due to limited number of each class of pod, a part of data was not available for the analysis. For the analysis of the lower position, data on 1-seeded, 2-seeded, 3-seeded and 4-seeded classes of thirty-eight inflorescences were used. Means and standard errors were calculated for each class. F-test was performed to examine the significance of the difference among the classes. For the analysis of the upper position, only data on 1-seeded, 2-seeded and 3-seeded classes of forty-four inflorescences were used because of lower seed set. Means and standard errors were calculated for each class and F-test was performed. Finally, we compared seed size between 2-seeded and 3-seeded classes. The percentage of average seed size of 2-seeded class to that of 3-seeded one was calculated for each inflorescence. Frequencies of lower than 80%, 80-120%, and higher than 120% of this percentage (three categories were tentatively determined) were calculated and then chi-square test was performed to examine the significance of the difference between the 2-seeded and 3-seeded classes. The reason of selecting these two classes is that they had sufficient number of pods.

Results

1. Within-inflorescence variation in seed set

Pod number, seed number and seed weight (pooled over all seeds contained) per inflorescence were 65.1 on average (standard error (SE) 15.9), 127.3 (SE 48.0) and 51.0 mg (SE 29.2), respectively. Average pod set was 84.4% (SE 13.7). Seed number per pod (i.e., seed set) and seed size (i.e., average seed weight) were 1.96 (SE 0.51) and 0.401 mg (SE 0.115), respectively.

Seed set was considerably different among pods. Of a total of 3265 pods pooled over fifty inflorescences, pod number of 0-seeded class was 515 (15.8%), 1-seeded 672 (20.6%), 2-seeded 967 (29.6%), 3-seeded 755 (23.1%), 4-seeded 303 (9.3%), 5-seeded 43 (1.3%) and 6-seeded 10 (0.3%). The percentage of the pods containing three or less was 89.1%. Each inflorescence showed a similar pattern of the variation in seed set to this (Fig. 1). It generally decrease upward along the inflorescence axis with large oscillation. This variation was partially attributable to pod position effect. A comparison between the upper and lower positions showed that the upper position had significantly poorer seed set than the lower position ($F=20.46$, $P<0.01$; Fig. 2) ; The upper position had 1.58 seeds per pod on average and the lower position 2.10 seeds. A similar trend was observed for seed size ; The upper position showed 0.339 mg of seed weight on average and the lower position 0.424 ($F=11.24$, $P<0.01$; Fig. 2).

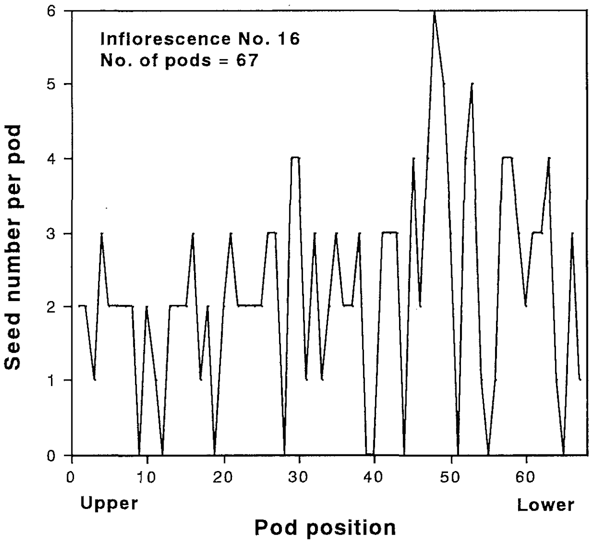

Fig. 1. Within-inflorescence variation in seed set. Data are on inflorescence no. 16. Pods are arranged in order of their position on the inflorescence.

2. A relationship between seed set and seed size

No difference in seed size was observed among the pod classes, except for 1-seeded class (Fig. 3). Seed size of 1-seeded class was slightly larger than the other ones. A detailed examination of the data showed that a moderate variation in a relationship between seed set and seed size exists ; Some inflorescences showed an increase of seed size with increasing seed set, whereas many inflorescences showed a constant seed size among the classes (Fig. 4). The percentage of 3-seeded to 2-seeded class (i.e., the ratio of the average seed size of 3-seeded class to that of 2-seeded one) differed between the upper and lower positions. In the lower position, the case of the percentage $<80\%$ was three, 80–120% was thirty-two, and $>120\%$ was only three. In contrast, in the upper position, it was five, twenty-five, fourteen, respectively. The percentage of $>120\%$ was significantly higher in the upper position than in the lower position ($\chi^2=8.13$, $P<0.05$).

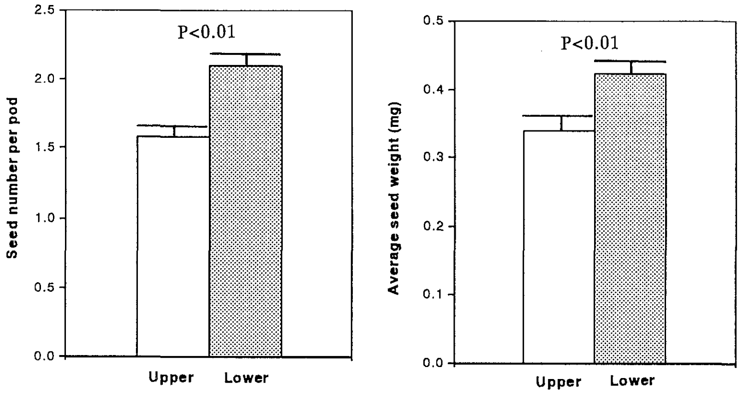

Fig. 2. Differences in seed number per pod and average seed weight between the upper (□) and lower (▩) positions of pods. N=50. Mean±SE.

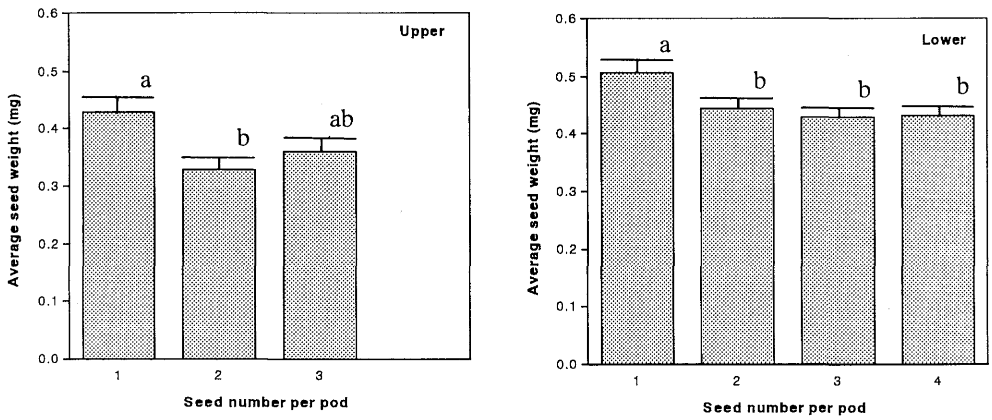

Fig. 3. Relationships between seed number per pod and average seed weight of white clover. Mean± SE. Means with the same letter are not significantly different at P=0.05.

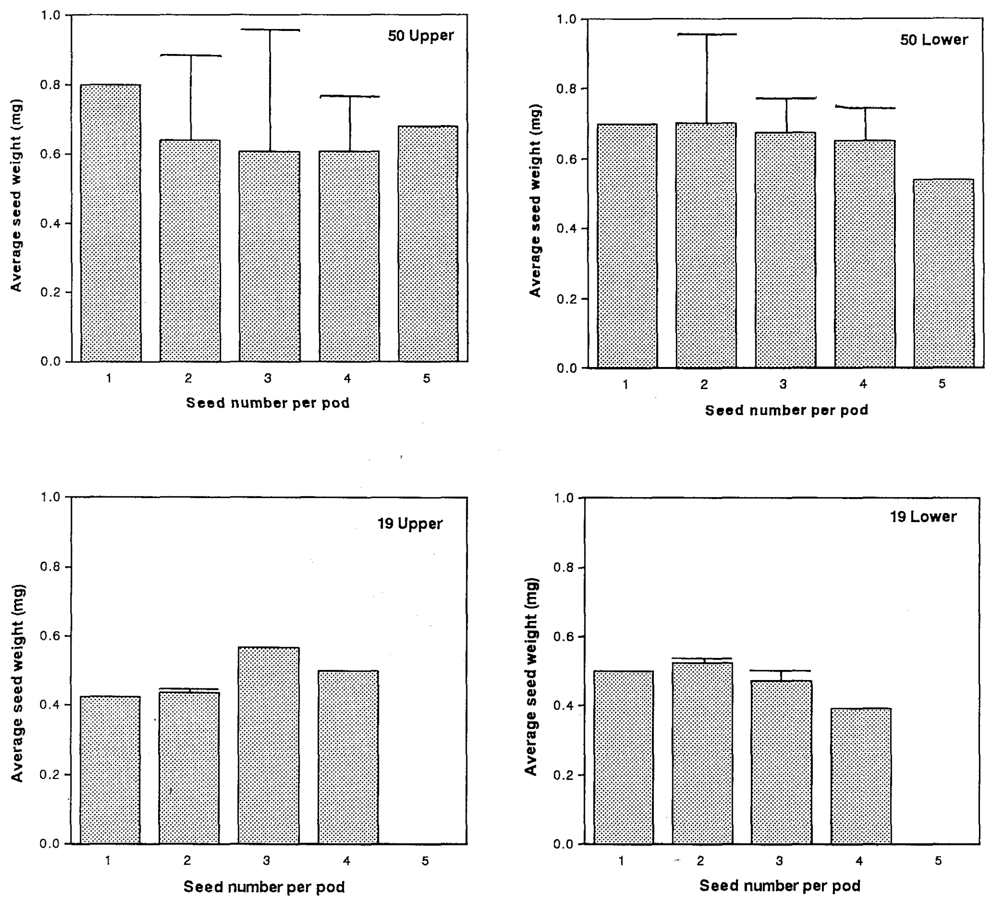

Fig. 4. Relationships between seed number per pod and average seed weight in the upper and lower parts of two representative inflorescences (no. 50 and no.19). Mean±SE. No statistical analysis was performed due to small sample sizes.

Discussion

1. Within-inflorescence variation in seed set

White clover is characterized as low seed set on average and varying seed set within inflorescence. Agronomic problems related to low seed set has been well studied, because it is a serious problem for seed production[4,7,8]. However, little attention has been paid for varying seed set. This paper is the first one about the pattern of within-inflorescence variation in seed set in fields.

A very interesting question is why florets of white clover set seeds at varying rates. Assuming that there is little variation in ovule number per pod, possible reason for this is three-fold : pollen limitation, resource limitation and climatic variability. Pollen limitation hypothesis includes both the behavior of pollinators and pollination frequency. Marshall *et al.*[3] pointed out the importance of the behavior of honeybees in varying seed set. A honeybee is likely to insert its head into individual florets in sequential order on the same inflorescence, when it visits an inflorescence (Sawada, personal observation). The floret which was firstly pollinated (within the inflorescence) may receipt more amount of heterogeneous pollen than the florets which pollinated later do. It may show a high seed set. In contrast, the latter floret may receipt more amount of self-pollen due to the carrying effect. It may have a high probability of the occurrence of geitonogamy, probably showing a low seed set. On the other hand, Rodet *et al.*[5] suggests the importance of pollination frequency. They suggested that a floret of white clover does not necessarily success seed set by one pollination, and it needs multiple (twice or more times) pollination to fertilize perfectly. If this is true, seed set would be modelately variable within inflorescence. Rodet *et al.*[5] also described that each floret begins to release its pollen before pollination, and that no negative load due to self-pollen occurs.

Resource limitation hypothesis includes (1) competition between florets of the same inflorescence due to difference in flowering time, (2) competition between inflorescence and stolon, and (3) selective investment by a mother plant. The former two factors (and climatic variability hypothesis) may cause a large-scale variation in seed set within inflorescence (i.e., a variation among cohorts of pod). They, however, may not cause a small-scale variation in seed set.

Selective investment hypothesis, in contrast, may account for this phenomenon. Selective investment refers to the situation where a mother plant invests more amount of resource to superior offsprings[1]. It has been reported for other legume species, e.g. *Lotus corniculatus* and *Phaseolus coccineus*[1]. For white clover, it has been well known that it aborts ovules or seeds frequently[2,3,7]. We, however, have no direct data supporting this hypothesis yet. It requires further studies to be confirmed. Several factors may affect the variation in seed set in a complex manner.

2. A relationship between seed set and seed size

White clover plants set seeds at highly varying rates. Seed set rate differs among florets even at near sites of the same inflorescence. This paper examined the consequence of this phenomenon for the seed size variation within inflorescence. Here, we attempted to test the following possible relationships : (1) Seed size is constant regardless of the seed number per floret (i.e., seed set) (relationship 1). (2) Seed size decreases with an increase in seed set (relationship 2). (3) Seed size increases with an increase in seed set (relationship 3). If white clover is likely to regulate to produce the same sized-seeds, we would observe the relationship 1. If white clover invests the same amount of resource into each floret regardless of the status of its seed set, we would observe the relationship 2. Resultantly, seed size decreases with an increase in seed set. If florets with higher seed set are likely to produce more superior offsprings than those with lower seed set, we may observe the relationship 3. This is the case of the selective investment[1]. The selective investment hypothesis explains the relationship 3 as follows : A floret receiving a limited amount of pollen has a low probability of being fertilized with good pollens. It, therefore, exhibits poor seed set, and the seeds are likely to be inferior. In contrast, a floret receiving a large amount of pollen has a high probability of being fertilized with good pollens. It exhibits almost full seed set, and the seeds are likely to be superior. If a mother plant invests more amount of resource to superior seeds, the relationship 3 would be detected.

Our data supported the relationship 1. Especially, in the lower position of pod, seed size was almost constant among the pod classes except for one-seeded one. Thus, it is strongly suggested that white clover regulates to produce a constant sized-seeds. This means that it maximizes the number of seeds with optimal size[1].

Interestingly, our data about the upper position of inflorescence showed that 3- seeded pods often produce larger seeds than 2-seeded pods within the same inflorescence. The upper florets generally flower and develop their seeds later than the lower florets, so that the formers are likely to be resource-limited[3]. Thus, the upper florets generally set lower number of seeds and produce smaller-sized seeds than the lower florets. These facts combined with our data suggest the possibility of selective investment of resource in the upper florets of an inflorescence. To confirm this possibility, we need further research including a pollination experiment with manipulation.

Acknowledgments

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要　　旨

澤田　均・畑中裕文・福田栄紀*・山下雅幸 (2000) : シロクローバの結実の頭花内変異とその種子サイズ変異への帰結. 日草誌 **45**, 329-334. 静岡大学農学部 (421-8529 静岡市大谷 836) * 東北農業試験場 (020-0123 盛岡市下厨川赤平 4)

シロクローバは多数の小花が集合した頭花をつけるが, 同じ頭花でも小花によって結実数が異なる。本研究では野外集団における結実の頭花内変異の様相とその種子サイズへの意味を調べた。その結果, 野外集団でも頭花内の結実変異が著しいことを確認した。この変異の一部は小花の着生位置で説明できた。

頭花上部の小花は下部の小花より結実率が低く, 種子サイズも小

さい傾向にあつた。一方，隣り合う小花の間ですら結実率が著しく異なった。この結実変異と種子サイズの関係を分析した結果，頭花下部で小花当り結実数とは無関係に均一サイズの種子をつくる傾向の強いことが示唆された。一方，頭花上部の小花では資源の選択投資の可能性があった。

キーワード：結実，種子サイズ，シロクローバ，頭花内変異.