Acta Bot. Neerl. 46(2), June 1997, p. 219–223

SHORT COMMUNICATION

Difference between the male and female components of fitness associated with the gene Ac in *Trifolium repens*

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SUMMARY

Trifolium repens is a species polymorphic for cyanogenesis. The polymorphism is caused by variation in two genes: *Ac* regulates the presence/absence of the cyanogenic glucosides linamarin and lotaustralin; *Li* is the structural gene for linamarase. Both genes also affect vegetative and reproductive characters of the plant. The female reproductive fitness of *acac* plants is about double of that of *Acac* plants. The male reproductive fitness was estimated by the proportion of *Ac*- and *Li*-plants in the progeny of *acaclili* plants in an experimental population with known frequencies of the cyanotypes. A significant excess of *Acac* plants was found in the progeny. The difference in male and female fitness is one of the many genetic and environmental factors that regulate the polymorphism for cyanogenesis that is characteristic for most populations of *T. repens*.

Key-words: cyanogenesis, polymorphism, reproductive fitness.

INTRODUCTION

In hermaphroditic plants reproductive fitness should be divided into male and female components. In many studies these components have been assumed equal. I report in this paper a clear case of non-equisexual reproduction in *Trifolium repens* and will discuss the effect of the difference on the maintenance of the cyanogenic polymorphism. The polymorphism is caused by variation in two genes: *Ac* regulates the presence/absence of the cyanogenic glucosides linamarin and lotaustralin; *Li* is the structural gene for linamarase (Oxtoby *et al.* 1991). Only plants with at least one active allele of Ac and Li liberate HCN when damaged. Such plants, called cyanogenic, are relatively protected against grazing by molluscs and possibly also by insects. Plants that possess only cyanoglucosids are protected against herbivores that have β-glucosidases in their gut, like molluscs (Kakes 1989). The genes *Ac* and *Li* have associated effects on the vegetative and reproductive characters of the plant (Kakes 1989, 1990). The most pronounced effect is that on flower and seed production: *Acac* plants produce only half of the flowers and seeds compared to acac plants. The allelic frequencies of the genes *Ac* and *Li* in natural populations will be influenced by the effects of fitness of the associated characters as well as by the primary effects of the genes.

Table 1. Distribution of genotypes in the progeny of four centrally located acaclili plants from an experimental population of *T. repens*

Family number	Genotype AcacLili	Acaclili	acacLili	acaclili	Total
1	6	38	25	74	143
2	7	9	8	38	62
3	3	15	12	27	57
4	9	14	14	29	66
Total	25	76	59	168	328

T. repens is a monoecious species with bisexual flowers. It has a gametophytic self-incompatibility system and is pollinated by bees and bumblebees. The production of flowers will influence both male and female fertility. In an earlier study (Kakes 1989) only female fertility was considered. In the present paper male fertility is studied by examining the progeny of *acaclili* plants produced in an experimental plot with known frequencies of the *Ac* and *Li* alleles. Each dominant allele of *Ac* or *Li* in such a progeny results from successful fertilization with an *Ac*- or *Li*-bearing pollen-cell.

MATERIALS AND METHODS

Ten plants of each of the four cyanotypes were taken from a backcross *AcacLili* × *acaclili*. Two cuttings of each plant were rooted in the greenhouse. The 80 rooted cuttings were transferred to the experimental garden of the Free University in a completely randomized plot with a plant distance of 60 cm (Kakes 1989). The seeds of four centrally located plants of the *acaclili* cyanotype, harvested twice a week over the flowering season, were used to raise four families with a total of 328 plants. The cyanotype of the plants was determined according to Kakes (1991). Pollen counts: the flowers of 12 plants, six *Acac* and six *acac*, were collected before anthesis and dried at 54°C for 24 hours. The anthers were macerated in 50 μl HCl/ethanol (1 part HCl 37%+2 parts ethanol 70%) for 15 minutes at 54°C and subsequently sonicated for 9 minutes. The suspension was neutralized with 1·67 N NaOH and brought to a volume of 200 μl. The pollen cells were counted with a Bürker Türk cell counter (W. Schreck, Hofheim/Is, BDR). For each count five flowers of one inflorescence were used.

RESULTS AND DISCUSSION

The cyanotypes of the four families are shown in Table 1. There is no significant difference between families, both for Ac (χ^2 1·249, d.f. 3) and *Li* (χ^2 4·197, d.f. 3). The conclusion is that the pollen constituting the male contribution originated from one pollen pool, in other words that the four female parents shared one neighbourhood, formed by the 80 plants in the plot. Therefore the combined results of the four families were studied, assuming that they all received their pollen from plants within the plot. The unweighted frequency of *Ac* and *Li* pollen in this pool is calculated as follows: *Acac* and *acac* plants were present in the parents in the proportion 1:1. The same is true for

Table 2. Comparison of all progenies with two models. Model 1 assumes equal contribution of all plants. Model 2 assumes a contribution weighted by the mean inflorescence weight of each cyanotype

		Model 1:				
Found:		Expected (1:3)			Goodness of fit:	
Acac	acac		Acac	acac	chi square	p
101	227		82	246	5·87	0·015
		Expected (1:3)				
Lili	lili		Lili	lili		
84	244		82	246	0·065	0·799
		Model 2:				
Found:		Expected (0·167:0·883)			Goodness of fit:	
Acac	acac		Acac	acac	chi square	p
101	227		55	273	46·83	<0·001
		Expected (0·225:0·775)				
Lili	lili		Lili	lili		
84	244		74	254	1·819	0·177

the frequency of *Lili* and *lili* plants. Assuming that all plants contributed equally, the proportion *Ac*:*ac* and that of *Li*:*li* pollen is 1:3. Table 2 shows that there is a significant excess of Acac plants, whereas the proportion of Lili plants is as expected.

To calculate the weighted contribution of the pollen plants the dry weight of the ripe inflorescences, published earlier, were used (Kakes 1989). These inflorescences were harvested twice a week over the flowering season. The pollen frequencies calculated from the mean weight of the four cyanotypes are given in Table 2. The frequency of *Ac* plants differs widely from this expectation. The frequency of *Li* plants fits the weighted model well, as expected: the inflorescences did not differ significantly between *Lili* and *lili* plants.

My conclusion is that the production of more flowers and more seeds by *acac* plants did not result in an overproduction of *acac* plants in the next generation. On the contrary, a small but significant excess of *Ac*-plants was found. In other words, there is a clear difference in male and female reproductive fitness in the hermaphroditic flowers of *Acac* and/or *acac* plants. What could be the cause of this difference? One or more of the following factors might play a role:

1. *Acac* plants may produce more pollen than *acac* plants.
2. Pollen from *Acac* plants may be transferred more efficiently than that of *acac* plants.
3. *Ac* pollen may be more successful in fertilization than *ac* pollen.
4. *Acac* zygotes may have a better chance to survive to young plants.

To test assumption 1 the number of pollen cells were counted in 54 flowers of *Acac* and *acac* plants. The results are given in Fig. 1. Although the pollen count of *Acac* plants is higher, Table 3 shows that the effect of genotype is not significant. Of course, this does not rule out the possibility that a difference in pollen production is part of the explanation. It could not be the whole explanation as the presumed difference in pollen production does not counteract sufficiently the difference in flower production.

I tried to test assumption 2 by counting the visits of pollinators (bumblebees and bees) to the different cyanotypes. A preliminary experiment did indeed show a significant preference for Acac plants, but the results were not confirmed in a later experiment. However, a difference in the behaviour of pollinators remains a (somewhat remote)

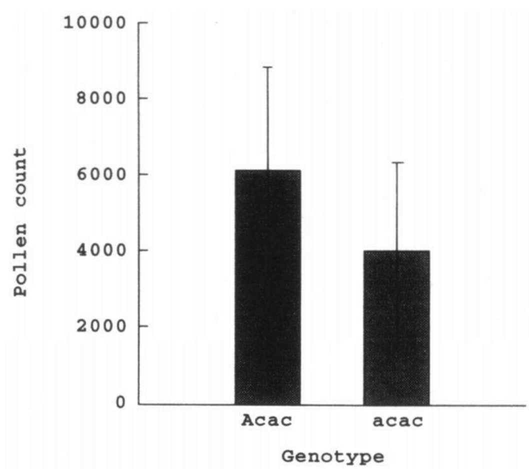

Fig. 1. The mean number of pollen-cells per flower, with standard deviation of Acac and acac plants.

Table 3. Nested analysis of variance of the mean number of pollen per flower

Source	Sum of squares	d.f.	Mean square	F-ratio	P
Between cyanotypes	44038100	1	44038100	2·561	0·141
Among plants within cyanotypes	171963000	10	17196300	3·966	<0·001
Among *acac* plants	54602900	5	10920600	2·519	0·044
Among *Acac* plants	117360000	5	23479000	5·413	0·001
Error	18211100	42	4335966		

possibility. Assumptions 3 and 4 can be safely ruled out because they would have shown up in some of the numerous controlled crossed performed by the present author and many others.

Apart from a difference in pollen production mentioned above, the most likely explanation for the difference in male fertility between *Acac* and *acac* plants is thus a difference in flower attraction and/or a difference in fertilization efficiency between *Ac* and *ac* bearing pollen. The question of how male fertility increases with the number of flowers on a plant has been addressed several times in the past few years (de Jong *et al.* 1992; Klinkhamer *et al.* 1993). The general conclusion of the authors is that the increase in male fertility is constrained by what we may call the law of diminishing returns: the higher the number of open flowers is at any moment, the more pollen is transferred between flowers of the same plant (geitonogamy). This effect lowers the amount of pollen that is exported to other plants. It may well be that this effect partly explains the lower male fertility.

The difference in male and female fertility is one of the many factors that influences the fitness of the cyanotypes of *T. repens*. It is not surprising that natural populations of *T. repens*, even in close vicinity, exhibit strikingly differences in the frequency of these cyanotypes.

ACKNOWLEDGEMENT

The author gratefully acknowledges the assistance of Dr J.J.M. Bedeaux with the statistical treatment of the data.

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