

Seeds of karaka (*Corynocarpus laevigatus*) cached by a rat

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The photograph (Fig. 1) was taken in July this year at 5 Ward Terrace, Sandringham, Auckland City, and shows a group of karaka kernels by a rat-hole near the property's compost-bin. The brittle husk (endocarp) of each kernel has been chewed open and the seeds are completely missing.



Fig. 1. Karaka kernels cached by rat. Pencil approx. 130 mm long. Photo: R.O. Gardner, July 2011.

There are several old karaka trees close by. I am inclined to think the cache was made from the trees' current (summer) crop, if only because there is no great accumulation of kernels under the trees themselves. Presumably the fruits fall to the ground, the fleshy, orange (non-poisonous) mesocarp erodes away or gets eaten by rodents, and the seeds gradually lose their poisonous substance (a nitrotoxin) through desiccation and water-logging. Because karaka very readily establishes in and around parent stands (at least, through the germination of fresh seed) this loss of immunity to predation would seem likely to be more important to the rat than to the tree.

Until recently the affinity of the Corynocarpaceae was unclear, but DNA evidence now puts the family in Cucurbitales, a placing hardly suggested by its morphology. Even more remarkably, its closest relative there is the Coriariaceae, plants which also contain a deadly (but chemically unrelated) substance. One has to suppose a coincidence here, such as is bound to appear now and again in a huge mass of data -- no need to postulate a "Toxic Terrane" in the biogeographical history of Zealandia.

Unequal sex-ratios in *Geniostoma ligustrifolium* var. *ligustrifolium* (Loganiaceae) – a genetical interpretation

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[submitted posthumously by his family]

Introduction

Geniostoma ligustrifolium A.Cunn. (Maori: *hangehange*) is a small bushy tree, 1-4 m tall when reproductive, with numerous, pale greenish-yellow flowers 4-5 mm in diameter. No work on the breeding system was known to the author prior to his previous paper (Rattenbury 1980) apart from: Bureau (1856) in a study of *Geniostoma* and *Logania*, (including *G. ligustrifolium*), states "that these plants are not only unisexual, but dioecious" and "the males appear to me much more frequent" (my translations); and Valetton (1902) in an examination of ten *Geniostoma* species (including *G. ligustrifolium*) concluded that all were gynodioecious.

Polliniferous and female *G. ligustrifolium* flowers at anthesis are very similar in appearance at low magnifications, especially when the latter have already been pollinated. Because putative hermaphrodite plants cannot be distinguished from males in the field, the term "male(s)" will be used for polliniferous plants at large. Females develop staminodes - similar to the stamens with their precociously-discharged (empty) anthers in open, pollen-bearing flowers (Fig. 1). "Male" flowers, themselves, contain well-developed, though somewhat smaller, gynoecia. For these reasons, the gender status of the genus has often been mistaken for hermaphrodite, i.e. flowers perfect (Cheeseman 1925, Allan 1961, Eagle 2006).



Fig. 1. Pollen from ten anther locules on "male" stigmata (c.15 x).

Rattenbury (1980) reported that plants of *G. ligustrifolium* are either pollen-bearing or seed-bearing, that is, the species is functionally dioecious, probably basically gynodioecious. He also described a process of secondary pollen-presentation in which the anthers in flowers of "male" plants open introrsely immediately prior to anthesis, depositing a thick coating of pollen on their own stigmata (Fig. 1). This appears to act as a barrier to pollination in the self-sterile hermaphrodite flowers (Fig. 2), with virtually complete absence of seed-set. Stigmata bear glandular hairs which are thought to produce an adhesive (Garnock-Jones & Endress 1997).

This article reports a study carried out from between 1978 and 2006, of several Auckland natural populations of *G. ligustrifolium* in order to determine the ratio of polliniferous to seed-bearing plants.

Materials

Materials used were almost entirely plants from naturally-occurring populations of *G. ligustrifolium*, which were analysed as below. A few cuttings, grown from natural material collected by Auckland University Grounds staff from local bush at Leigh Marine Station, were also used. These included "male" and female plants and were used for studies of seed abortion in artificial crosses (see below).

Methods

Sex-ratios in some natural Geniostoma populations

Populations Studied

Ten natural populations, that were fairly thoroughly examined, are listed in Table 1.

Population structure

Populations often tend to be small, mainly because the disturbed areas in which they become established are usually rather narrowly circumscribed and perhaps

because the distribution of seeds, mainly by small birds, is often restricted to small, open areas. As can be seen from the last column of Table 1, all the populations studied have been established in open or disturbed ground where the soil has been exposed at some time.

Seed-dispersal is usually by small birds such as native bellbirds (*Anthornis melanura*), silvereve (Zosterops *lateralis*) and others (Gaze & Fitzgerald 1982, Anderson 1997). The capsules, when mature, split open, exposing the seed-mass in a gelatinous placental matrix (Allan 1961) which is attractive to birds (Anderson 1997). Seeds in bird droppings are deposited, sometime after ingestion by the bird, potentially at considerable distances from, and probably only occasionally beneath, the source plant. Seeds which fall from the parent plant remain for some time in the swollen placental tissue and do not germinate (Burrows 1998).

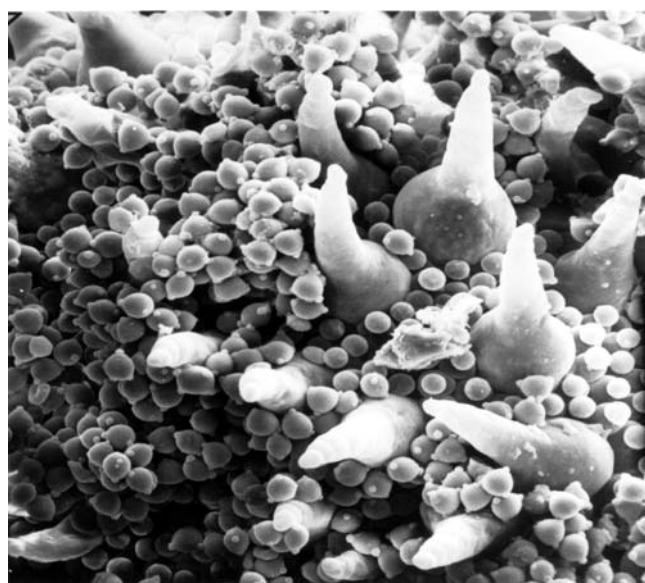


Fig. 2. Massive pollen deposition on "male" stigma – a barrier to cross-pollination (250 x).

Anderson (1997) has shown that seeds germinate after passage through a bird and also if they are extracted from the placental matrix and washed. Burial and subsequent disinterment results in germination, even after several years (Burrows 1998), presumably after the breakdown or leaching away of the inhibiting factor in the placental matrix. This is an example of *autoallellopathy* in which an inhibiting substance from the parent plant prevents its own seed from germinating until removed by passage through a bird, mechanical removal and washing of seeds or burial.

Despite this limitation on regeneration in any given population, many, if not most, natural populations will be composed of plants from more than one cross either by repeated seeding by resident bird populations from other seed sources or by delayed,

Table 1. Populations studied (Waitakere Ranges and Auckland City), with ratios of "male" plants to female and terrain types. Significantly different from equality at: ^ 10% level or higher; * 5% level; ** 2% level; * 1% level.**

POPULATION	"MALE"	FEM.	TOTAL	% FEM.	X ²	TERRAIN
1. Anawhata Hut	26	13	39	33	4.3*	old burn
2. Auckl. Domain	33	16	49	33	5.9**	city park
3. Hillsborough	45	27	72	38	4.5*	roadside
4. Home Track	53	37	90	41	2.8^	bush track
5. Huapai Reserve	110	76	186	41	6.3**	forest gap
6. Mercer Bay Trk	108	94	202	47	0.1^	bush track
7. D.McLean Trk	164	127	291	44	4.7*	bush track
8. Pararaha Cliff	47	26	73	36	6.0**	land slip
9. Turanga Road	100	64	164	39	7.9***	old farm
10. Zion Hill Track	34	29	63	46	0.4^	bush track

germination of buried seeds triggered by some seasonal or other natural cause. As can be seen below and in the Discussion, most of the populations used in this study probably fall into the category of mixed origin, giving "male" : female ratios intermediate between 2:1 and 1:1. The small populations 1 and 2 which are so nearly 2:1 may be exceptional in arising from a single parental cross in a single seeding or more than one seeding from the same source plant.

Results

It is significant that there are more "males" than females in all populations, supporting the argument that the ratios in all the populations are not simply random departures from equality. Otherwise more females than "males" should sometimes occur. As far as possible, all plants in a population were recorded for the sex of their flowers.

Evidence for trioecy from field and laboratory studies

Evidence for self- and cross-incompatibility in "males"
Whether the pollen barrier acts in a purely mechanical way or presents a biochemical block to pollen of other plants is not known. There was no sign of penetration of pollen-tubes into the specifically-stained styles of "males", although germination on their stigmata, was regularly observed.

Evidence for the existence of hermaphrodite plants (MmFF and MmFf)

Because ovules in hermaphrodite plants are rarely fertilised (the pollen barrier) they can only exceptionally be distinguished from true males by capsule production. At the microscopic level apparently-normal ovules were counted in 110 "male" gynoecea (ten flowers each from eleven, probably hermaphrodite, plants) from population 4, with an average of 41.1 ovules per ovary, as compared with 160 female gynoecea (ten flowers each from sixteen plants) from the same population, with an average of 49.1 ovules per ovary. This may show a potential for viable seeds in hermaphrodites, the smaller numbers possibly reflecting the expenditure of resources on pollen production.

The production of capsules on "male" plants, although very rare, points to the existence of functional hermaphrodites among their flowers. These fruits could have arisen from occasional penetration of the pollen barrier by pollen from other plants, perhaps through failure of an anther locule to discharge its contents or brushing away of the pollen barrier by the pollinator. In one exceptional plant, eight seed-pods, arising from among hundreds of "male" flowers, were found to contain 35 seeds (one abortive) averaging 4.25 per capsule. As normal seed content in fruits of

Table 2. The six possible genotypes and their sex and viability (MM can't exist).

GENOTYPE	SEX	POLLEN	OVULES	SEEDS	FUNCL. SEX
MmFF	hermaphrodite-1	viable	viable	very rare	Male
MmFf	hermaphrodite-2	viable	viable	very rare	Male
Mmff	male	viable	abortive	none	Male
mmFF	female-1	none	viable	viable	Female
mmFf	female-2	none	viable	viable	Female
mmff	neuter (abortive)	none	none	none	None

females is usually between 40 and 60 per capsule, this gives further support to the argument for the rarity of pollen-tube penetration in hermaphrodite plants.

The two postulated hermaphrodite genotypes (in Table 2) were indistinguishable and could only be identified by extensive inter-breeding.

Evidence for true males (Mmff)

The existence of true male plants is more difficult to demonstrate than of hermaphrodites. As can be seen in Table 3, if the genetic model is correct, they will be relatively few among offspring of crosses four and six (1 in 8 and 1 in 4, respectively) and absent entirely from the other four.

Attempts to cross-pollinate the "males" from which the tiny petals, with their attached stamens, were removed just prior to anthesis, or from which pollen was removed from the stigmata after anthesis, proved unsuccessful. This would have distinguished true males from hermaphrodites and demonstrated the existence of both kinds of "male" more conclusively.

Two "male" plants of five from population 2 contained withered or blackened ovules (abortive) and were possibly Mmff genotypes. Although breakdown of ovular tissue with age cannot be ruled out, no evidence was seen, in the ovaries or elsewhere, of breakdown of tissues other than ovules. In nine of 22 "males" examined from the same population, the ovules were detectably smaller than in the others, possibly indicating their inviability in true males. In both the above cases, the numbers are not significantly different from equality ($X^2 = 0.4$ and 0.7) as expected in the progeny of cross 6.

The existence of populations with "male": female ratios of 2:1 would only come about in crosses involving true male plants, with this and other models considered in the Discussion.

Evidence for females (mmFF and mmFf)

Female flowers are readily distinguished from "males", especially at anthesis, by their pollen-free stigmata and their undifferentiated staminodes where there is

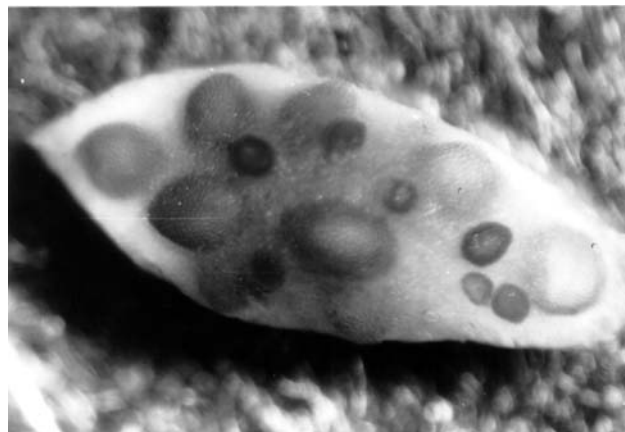


Fig. 3. One placenta showing varying degrees of abortion in ripening seeds.

no evidence of the ruptured pollen sacs seen in Fig.1. All of the many female plants examined produced large numbers of capsules.

Evidence for neuters (mmff)

Neuters should produce no pollen and, at most, only abortive ovules or seeds. If abortion were prevalent in some populations (where crosses 4 and 6 were involved) seed number would be expected to be lower than average. This reduction in seed number might be established by reference to "normal" seed number, but seed numbers vary considerably within and between populations, whether or not neuters are considered to be present, mainly because of failure of all ovules to be fertilized. This is amply borne out where seed content is often very different (up to three times) in the two locules of the same ovary. Seed abortion seems usually to occur soon after fertilization but occasionally it can be observed among maturing seeds in the ripening fruits (Fig. 3).

Several cuttings, from mature "male" and female plants collected and potted at Leigh Marine Laboratory, were stored in a row in the University of Auckland nursery, virtually in contact with one another. These flowered the following season and the females set seed. The four females had varying numbers of aborting seeds, these being dark and variable in size (Fig. 3).

Table 3. The six possible crosses and their offspring from the five functional genotypes

CROSS	H-1	H-2	M	F-1	F-2	Abort.	"M" : F	% FEM
1. H-1 (MmFF) x F-1 (mmFF)	1	-	-	1	-	-	1:1	50
2. H-1 (MmFF) x F-2 (mmFf)	1	1	-	1	1	-	1:1	50
3. H-2 (MmFf) x F-1 (mmFF)	1	1	-	1	1	-	1:1	50
4. H-2 (MmFf) x F-2 (mmFf)	1	2	1	1	2	1	4:3	43
5. M (Mmff) x F-1 (mmFF)	-	1	-	-	1	-	1:1	50
6. M (Mmff) x F-2 (mmFf)	-	1	1	-	1	1	2:1	33

Table 4. Varying amounts of seed abortion in maturing placentae of four female plants. Departure of actual from expected is significant at: * 2%; ** 10%; levels. ^Not significant.

Plant no.	Fruits analysed	Aborted seeds	Normal seeds	% Aborted	% Expd. (in cross)	X ²
9	10	4	359	0.01	0 (1,2,3 or 5)	0.04 [^]
12	26	258	798	24.4	25 (6)	0.19 [^]
13	28	134	1158	10.4	12.5 (4)	5.37*
18	25	126	1012	11.1	12.5 (4)	2.13**

Table 4 shows the numbers of abortive and normal seeds present in fruits of the four females.

If abortive seeds are in fact neuters (mmff) the results fit reasonably well with predicted numbers of aborted seeds (Table 3, column 6) in the six crosses of the model. It seems likely that the very close proximity of the cuttings to one another would ensure that each female received pollen mainly from a single "male" plant next to it. Infrequent pollinations from more distant "males" could account for the abortion values being below expected, as in plants no.13 and 18.

As shown in Table 3, populations such as no.2, which, from its low numbers of females (c.33%), is most likely the result of cross no.6, should produce four genotypes (herm-2, male, fem-2 and neuter, in equal numbers). Viable crosses among these four will produce fruits with seed numbers that are reduced by 12.5% (herm-2 X fem-2) and 25% (male X fem-2) in about equal numbers (but none with unreduced numbers), these reductions resulting from the production of abortive neuters (in crosses 4 and 6, respectively). Plotting seed number in the plants of this population should produce a bi-modal distribution. In contrast, a population such as no.5 (Mercer Bay) with females close to 50% should show virtually no seed abortion and seed numbers should

give a single, roughly-normal distribution. Both these arguments are borne out by the graphs in Fig. 4. (When totals of each of the six plants analysed in the Domain population were examined separately, it became apparent that four plants were probably the result of cross 6 and two of cross 4 giving two unequal-sized, reduced distributions).

Discussion

A proposed genetic model

It is proposed that two recessive mutations, 'm' and 'f', in a putative ancestral hermaphrodite, have occurred on different chromosomes, such that the double-recessives, 'mm' and 'ff', cause complete suppression of pollen formation and abortion of ovules or young seeds, respectively. There was no indication of plants with 'mmff' genotypes, with flowers in which both sexes are sterile, occurring in the field, these not surviving beyond the immature seed stage, though flowerless specimens were (very rarely) observed in population 4, perhaps from causes unrelated to genes 'm' and 'f'. Several other models are examined below.

The proposed genetic explanation does not allow for a significant excess of females over males from any cross. This is in accordance with the population numbers in Table 1. However, it allows for 1:1 sex

Fig. 4. Seed numbers in two of the populations studied showing evidence of seed abortion in population 2 and its absence in population 5 (see text for explanation).

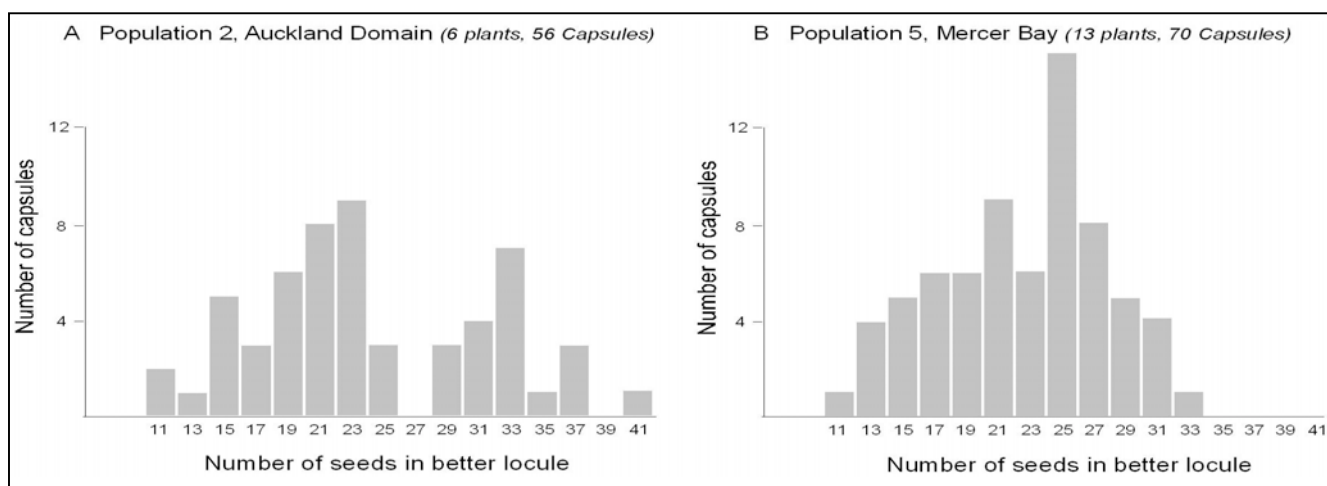


Table 5. Independent assortment v. linkage, dominance v. recessiveness, and their effects on sex-ratios. *Truly dioecious (Lewis 1941)

Mutant states	INDEPENDENT ASSORTMENT				LINKAGE			
Male development suppressor	DOM	DOM	rec	rec	DOM	DOM	rec	rec
Female development suppressor	DOM	rec	DOM	rec	DOM	rec	DOM	rec
Possible "M": F ratios	1:1 2:1	1:1 4:3 2:1	1:1 2:1	1:1 4:3 2:1	1:1 2:1	1:1* 1:1*	1:1* 1:1*	1:1 2:1

ratios in four of the six possible crosses. This may be the case in populations 5 and 9 which do not vary significantly from equality of sexes. Populations with ratios intermediate between 2:1 and 1:1 could result from cross 4, or more probably from mixed crosses, involving crosses 4 and/or 6 with the others in varying amounts.

Alternative Models

Other possible models were considered where the two genes for sex-expression were linked, and/or where either or both of these genes were dominant.

Table 5 summarizes the alternative states of the two genes and the possible ratios of the sexes in offspring derived from seeds of a single parent plant.

In most of these models, intermediate ratios between 1:1 and 2:1 in *whole populations* could arise from mixed crosses, as is likely the case in some of the populations studied. However, in the case of seeds from *individual crosses* (rather than whole populations) the variable production of neuters (ovule or seed abortion) as seen in the fairly good correspondence between observed and predicted in plants 12, 13 and 18 in Table 4, together with the evidence in the graphs of Fig. 4 showing two different levels of reduction in seed-production in population 2, suggests a model with no linkage, with the female determinant recessive and the male determinant either recessive or dominant, permitting ratios of male to female, from the offspring of individual plants, of 4:3.

While in theory there is nothing to chose between the two models which predict a ratio of four "males" to three females in the offspring of individual plants, the

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postulation of a model in which the male determinant is recessive would, on the principle of Occam's razor, be the better choice, as dominance for 'M' would probably require "selection of modifying genes or alleles" (Lewis, *op. cit.*) and would be of no selective advantage over both male and female being recessive suppressors (evolutionarily the more primitive condition). Weak linkage could also produce 'M' : F ratios intermediate between 1:1 and 2:1 but unlikely to be consistently 4:3. Again there seems to no need to add this complication in the choice of a preferred model.

Evolutionary considerations

The genetic mechanism postulated for *Geniostoma* may not be uncommon in the New Zealand native flora. Of 126 plants in a population of *Coprosma spathulata*, 42% were females, a figure comparable with some of the *Geniostoma* populations examined in this paper. Godley (1964) found that 10 out of 16 populations of N.Z. native species he examined showed a preponderance of males. Females in some ranged from about one-third to nearly one-half, as in *Geniostoma*.

The greater numbers of pollen-bearing plants in some gender-polymorphic species may be related to the seemingly-primitive evolutionary status of flower development in a flora where long-tongued native bees are absent, so that complex co-evolution of flower structure with pollinators is not highly developed, and pollination therefore more haphazard. Godley (*op. cit.*) suggests that "an excess of males may have arisen by selection to compensate for inefficiencies in pollination, in situations where seed production is limited by the availability of pollen and not by the number of ovules".