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HYBRIDIZATION OF PASSIFLORA EDULIS SIMS AND
P. EDULIS SIMS F. FLAVICARPA DEGENER

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SUMMARY

Passiflora edulis Sims and *P. edulis* Sims f. *flavicarpa* Degener were crossed using a bagging and emasculation technique. The hybrid *P. edulis* x *P. edulis* f. *flavicarpa* was intermediate to the parental forms in flowering season, flowering habit and in several morphological characters. The comparative ease of manual crossing of *P. edulis* and *P. edulis* f. *flavicarpa* indicated the close genetic relationship of the two forms irrespective of diversity. The presence of barriers to inter-form crossing and the normal meiosis of the hybrid suggested that speciation is occurring between *P. edulis* and *P. edulis* f. *flavicarpa* and the chromosomes of the two forms are not differentiated by major structural changes.

I. INTRODUCTION

The purple passionfruit is cultivated widely in both Australia and other countries. However, the golden or yellow passionfruit *Passiflora edulis* f. *flavicarpa* has achieved significance as the commercial passionfruit of Hawaii. The purple passionfruit and the yellow form differ in morphology, in flowering behaviour and in characteristics of major commercial significance such as disease-resistance, yield and fruit flavour.

Characteristics of value lacking in *P. edulis* have been incorporated by a programme of breeding and selection from *P. edulis* f. *flavicarpa* but without investigating the mode of origin and cytological relationship of the two forms. The planning of a more efficient breeding programme may be possible if this knowledge is obtained. The two forms were hybridized and a cytological and comparative study of the parental forms and their hybrid undertaken to further suggest their probable relationship.

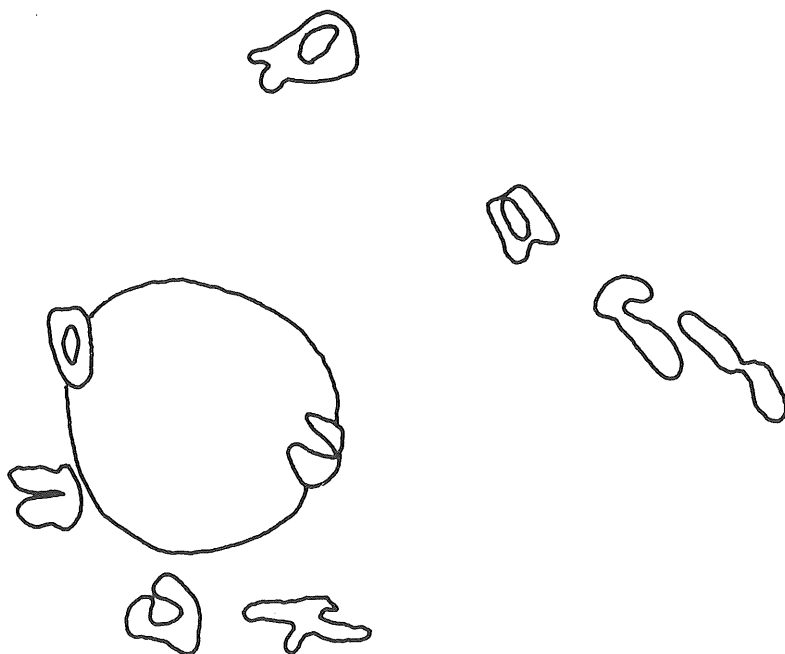


Fig. 1 (a).—*P. edulis* f. *flavicarpa*. Nine bivalents at diakinesis with two associated with the nucleolus (x 2700).

II. REVIEW OF LITERATURE

P. edulis and *P. edulis* f. *flavicarpa* differ in morphology, in flowering behaviour and in specific ecological requirements. *P. edulis* f. *flavicarpa* is yellow-fruited and generally larger-fruited; has more intensely pigmented flowers and stems and a more vigorous vine than *P. edulis*. In Hawaii, *P. edulis* flowers are reported as opening early in the morning around dawn and closing before noon whereas flowers of *P. edulis* f. *flavicarpa* open around noon and close about 9 or 10 p.m. (Anon. 1954). Also *P. edulis* f. *flavicarpa* is adapted to warmer conditions (Anon. 1954; Wills, Stephens and Groszmann 1961).

The two forms also differ in characteristics of major commercial significance including fruit-set, yield and disease resistance. *P. edulis* f. *flavicarpa* has a more extended cropping period and a higher yield than *P. edulis* (Kefford and Vickery 1961). However, self-incompatibility has been found in clones of *P. edulis* f. *flavicarpa* in Hawaii (Akamine and Girolami 1959) and in Queensland (Wills, Stephens and Groszmann 1961) while *P. edulis* is regarded as being self-compatible. It is apparent that low fruit-set problems could be associated with aspects of the allogamous regime. Stebbins (1950) states that chiasma frequency is correlated with the type of breeding system, low chiasma frequency being associated with high out-crossing or self-incompatibility. The basis of fruit-set problems in different types of passionfruit may be indicated by a knowledge of the levels of chiasma frequency which prevail.

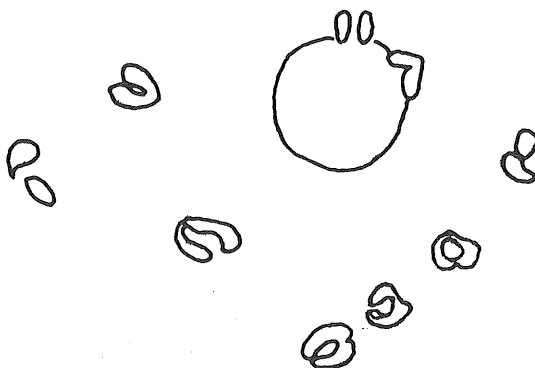


Fig. 1 (b).—*P. edulis*. Nine bivalents at diakinesis with two associated with the nucleolus ($\times 1250$).

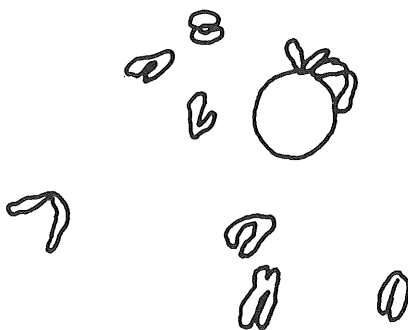


Fig. 1 (c).—*P. edulis* \times *P. edulis* f. *flavicarpa* F_1 . Nine bivalents at diakinesis with two associated with the nucleolus ($\times 1250$).

P. edulis is susceptible to fusarium wilt caused by a soil-borne organism *Fusarium oxysporum*. Commercial production declined in Queensland in the early 1950s because of the widespread occurrence of this disease. However, commercial use of *P. edulis* f. *flavicarpa* as a wilt-resistant rootstock for *P. edulis* has been made with success (Groszmann and Purs 1958).

The advantages of incorporating wilt resistance and other desirable characteristics into commercial *P. edulis* by a programme of breeding and selection are apparent. In south-eastern Queensland, the passionfruit industry depends almost entirely upon commercial hybrids of *P. edulis* and *P. edulis* f. *flavicarpa* (with resistance to fusarium wilt, tolerance to PWV, extended cropping period and higher yield) bred by Groszmann and Meurant at Redlands Horticultural Research Station and released to industry in 1959 (personal communication Groszmann and Meurant). Also, natural hybrids of the two forms are recorded in Queensland (Wills, Stephens and Groszmann 1961) and in Hawaii (Bowers 1953).

P. edulis and *P. edulis* f. *flavicarpa* both have the same chromosome number ($2n = 18$) and exhibit normal bivalent behaviour at P.M.C. Meiosis (Storey 1950). Cytological studies of the interform hybrid have not been recorded before the present study, although genetic studies have been reported by Nakasone, Hirano and Ito (1967). A more detailed study of P.M.C. Meiosis in the two forms and their hybrid may indicate further relationships.

Different modes of origin have been suggested for *P. edulis* f. *flavicarpa* by various workers. Pope (1935) suggested *P. edulis* f. *flavicarpa* had a hybrid origin and arose from a cross of *P. edulis* and another species, possibly *P. ligularis*. Storey (1950) disputed this on the grounds that the suggested species hybrid had normal bivalent behaviour. He suggested that, considering the differences in morphology and flowering characteristics between *P. edulis* and *P. edulis* f. *flavicarpa*, a drastic mutation was involved in their evolution. It has also been suggested that *P. edulis* f. *flavicarpa* as the mutant form originated in Australia (Anon. 1954).

III. MATERIALS AND METHODS

1. Technique of hybridization

Clones of *P. edulis* and *P. edulis* f. *flavicarpa* from Redlands Horticultural Research Station were selected as parents for the proposed cross which was made in the October-November period when the flowering season of the two forms coincided. Flowers of both forms were bagged with 102 mm x 152 mm paper bags 24 hours before opening to prevent insect pollination.

Crosses using f. *flavicarpa* as female parents were not attempted in this exercise because of absence of fruit set in a previous extensive series of artificial pollinations. All crosses were made at 9 to 10 a.m. The flowers of *P. edulis* were used as females and normally were open with receptive stigmas at that time. Flowers of *P. edulis* f. *flavicarpa* normally opened after midday but their protandrous nature ensured adequate pollen for crossing in the morning. The flowers of *P. edulis* were emasculated and pollen from the male parent brushed over the stigmas. The flowers were then rebagged to prevent further pollination or possible damage.

2. The F₁ hybrid *P. edulis* x *P. edulis* f. *flavicarpa*

P. edulis x *P. edulis* f. *flavicarpa* F₁ hybrid seedlings were planted 3 m apart in a row, trained over a two-wire horizontal trellis and grown to flowering. Vines of the parental clones were established for comparative studies at the same time as grafted plants on *P. edulis* f. *flavicarpa* rootstock. Flowering season for the *P. edulis* F₁ and the parental forms were determined by observations of presence or absence of flowering every two weeks, from August 1966 to October 1967.

3. Cytology

Flower buds were fixed in alcohol acetic acid for 24 to 48 hours and stored in 70% alcohol at 7°C. (Darlington and La Cour 1962). Anthers were macerated in aceto-orcein for staining. All easily visible pieces of tissue were removed and the P.M.C. were squashed after being heated to 60°C.

P.M.C. Meiosis was examined and the number of chiasmata per bivalent were counted at diakinesis of P.M.C. Meiosis in at least 10 cells of each form. Also the level of chiasma terminalization was assessed in *P. edulis*, *P. edulis* f. *flavicarpa* and their hybrid by expressing terminal chiasmata as a percentage of total chiasmata.

4. Morphological characters

Six characters differentiating *P. edulis* and *P. edulis* f. *flavicarpa* were examined in the hybrid as a further means of indicating the relationship of the parental forms. The characters examined were corona pigmentation, external fruit colour and lustre, and seed colour, shape and ornamentation. Seed shape and ornamentation were identified by drawing seed of the hybrid and parental forms to scale (figure 2).

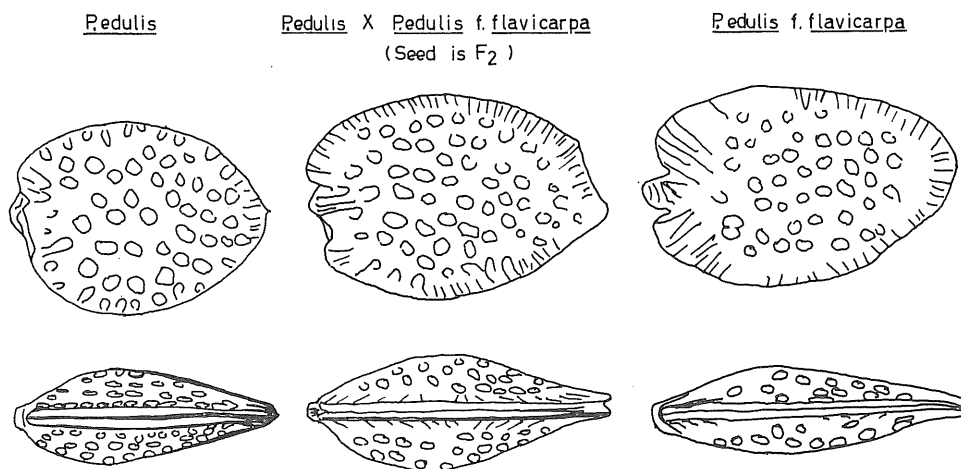


Fig. 2.—Seed shape and ornamentation. (Plan and side-elevation).

The expression of any of the selected characteristics was arbitrarily assigned a value of one in *P. edulis* and five in *P. edulis* f. *flavicarpa* on a scale of 0 to 6 to allow for the possibility of transgressive segregation. One of the seven possible values was then given to the hybrid on the basis of character expression relative to the parents.

IV. RESULTS

1. The hybridization

Eight crosses attempted between *P. edulis* and *P. edulis* f. *flavicarpa* provided four fruit and a total of 181 seeds. The 125 *P. edulis* x *P. edulis* f. *flavicarpa* F₁ seedlings obtained were morphologically normal and grew vigorously. Hybrid seedlings could be distinguished from *P. edulis* seedlings when 150 to 250 mm high by the more intense anthocyanin pigmentation of the stem and the leaf veins.

2. Flowering characteristics of the *P. edulis* x *P. edulis* f. *flavicarpa* Hybrid

Flowering season for the F₁ hybrid and parents is presented in table 1. These data indicated that *P. edulis* had two definite seasons of flowering, one over spring and early summer (July to November) and one of short duration over autumn and early winter (February to April). In contrast, *P. edulis* f.

flavicarpa had a single extended flowering season (October to June). The F₁ hybrid also had a single extended flowering season (October to May). *P. edulis* and *P. edulis* f. *flavicarpa* commenced flowering for the summer crop in July-August and in October-November respectively. The F₁ hybrid was intermediate, with the first flowers occurring in September-October. Winter flowering in *P. edulis* ceased in April whereas the flowering seasons of the F₁ hybrid and *P. edulis* f. *flavicarpa* were completed in May and June respectively.

3. Cytology

P. edulis (F₁) and the parental forms had the same chromosome number ($2n = 18$). Meiosis was normal in the F₁ hybrid and in the parental forms and the hybrid exhibited a lower chiasma frequency than either parent (figure 1, table 2). Also, the percentage of chiasmata which had terminalized at diakinesis was reduced in the hybrid.

TABLE 2
BIVALENT OCCURRENCE AND CHIAsMA FREQUENCY AND TERMINALIZATION AT P.M.C. MEIOSIS IN
P. edulis, *P. edulis* f. *flavicarpa* AND THEIR HYBRID

| — | No. Cells Examined | Total No. Bivalents | Mean Bivalents per PMC | No. Chiasmata per Bivalent | Percent Terminalization of Chiasmata |
|---|--------------------|---------------------|------------------------|----------------------------|--------------------------------------|
| <i>P. edulis</i> | 15 | 135 | 9 | 1.70 | 84.3 |
| <i>P. edulis</i> (F ₁) G2 | 10 | 990 | 9 | 1.54 | 60.7 |
| G7 | 10 | 90 | 9 | 1.61 | 59.6 |
| <i>P. edulis</i> f. <i>flavicarpa</i> .. | 13 | 117 | 9 | 1.80 | 85.6 |

4. Morphological characters

Ratings for six plant characters on the intensity or degree of development relative to that of the parents are presented in table 3. A description of each trait as expressed in the parents and their hybrid is also found in table 3.

Differences in seed shape and ornamentation in the F₁ hybrid and the parents are shown in the plan and side elevations in figure 2. The seed of *P. edulis* f. *flavicarpa* is rather flat and elongate and could be distinguished readily from the round and thicker seed of *P. edulis*. Also, the pattern of pitting on the testa of the seed differs between the two forms.

The F₁ vines differed slightly in fruit colour but not discernably in other traits. The expression of the six characters in the hybrid was generally intermediate between the parents. Fruit lustre is an exception as it has a similar expression in both the hybrid and *P. edulis* f. *flavicarpa*.

TABLE 3
MORPHOLOGICAL CHARACTERS IN *P. edulis*, *P. edulis* f. *flavicarpa* AND THEIR F₁ HYBRID

| | <i>P. edulis</i> | | F ₁ Hybrid | | <i>P. edulis</i> f. <i>flavicarpa</i> | |
|---------------------------|------------------|--|-----------------------|---|---------------------------------------|--|
| | Rating | Description | Rating | Description | Rating | Description |
| Corona pigmentation .. | 1 | Pale band of proximal pigmentation | 3 | Band of intermediate intensity and width | 5 | Dark purple band wider than in <i>P. edulis</i> |
| Fruit colour | 1 | Deep purple | 2-3 | Reddish purple | 5 | Pale yellow |
| Fruit lustre | 1 | Dull waxy | 5 | Vitreous | 5 | Vitreous |
| Seed colour | 1 | Black | 4 | Deep brown | 5 | Brown |
| Seed shape— Plan | 1 | Round—No indentation around funicle | 2-3 | Slightly elongate—funicular indentation obvious | 5 | Elongate—marked funicular indentation |
| Side elevation .. | | Broad elliptic | | Broad elliptic | | Narrow elliptic |
| Seed ornamentation .. | 1 | Pits uniform in size and well distributed over testa to margin | 3 | Intermediate size and distribution of pits over testa | 5 | Pits smaller than in <i>P. edulis</i> and centrally distributed in testa |

V. DISCUSSION

The cross *P. edulis* x *P. edulis* f. *flavicarpa* was made artificially without difficulty and the hybrid seed germinated readily producing morphologically normal seedlings. The ease with which the two forms were crossed, in spite of difficulties when f. *flavicarpa* was the female parent, is a further indication of their close relationship.

The difficulty involved in hybridization using f. *flavicarpa* as female parent was associated with pollen germination to fertilization stages. Pollen incompatibilities have been recorded in *P. edulis* f. *flavicarpa* in Hawaii and Queensland and this barrier to hybridization may be caused by pollen incompatibilities. However, this postulate remains to be tested.

Ecogeographical and physiological barriers to crossing are known to exist between *P. edulis* and *P. edulis* f. *flavicarpa* in the wild. *P. edulis* f. *flavicarpa* is adapted to warmer conditions than *P. edulis* (Anon. 1954; Wills, Stephens and Groszmann 1961). The flowering season and flowering habit of the two forms differ in Hawaii (Pope 1935) and also in south Queensland (table 1). However, the flowering season constitutes an incomplete barrier to crossing in south Queensland. *P. edulis* f. *flavicarpa* has one extended period of flowering which traverses the minor February-April flowering of *P. edulis* and shows partial coincidence with the latter part of the July-November flowering of *P. edulis*.

Differences in flowering habits are probably more significant as barriers to crossing between the forms than any differences in flowering season. In Hawaii (Anon. 1954) *P. edulis* flowers are reported as opening early in the morning around dawn and closing before noon, while flowers of *P. edulis* f. *flavicarpa* open around noon and close about 9 or 10 p.m. However, in southern Queensland, *P. edulis* flowers do not close before noon but remain open until 9 to 10 p.m.

In both forms, anther dehiscence occurred around dawn in the unopened flower, but pollen was normally shed and exploited by insects after the opening of the flowers. Functional pollination is probably complete by 9 a.m. in *P. edulis* and natural crossing between the different forms may be expected to be a rare occurrence. The occurrence of hybrids in the widely separated regions of Hawaii (Bowers 1953) and north Queensland (Wills, Stephens and Groszmann 1961) does not support this latter contention. However, the different conditions encountered in close commercial cultivation of these forms in Hawaii and Australia may well alter the natural level of inter-form crossing. Similar responses were recorded in *Phaseolus* by Crispin (1960).

The F₂ progeny of *P. edulis* x *P. edulis* f. *flavicarpa* were also generally quite fertile, although a 6 to 8% frequency of weak types of abnormal morphology and low fertility occurred. These abnormal F₂ plants had harsh-textured and curled leaves with prominent veining, and two instead of three carpels commonly developed in the few fruit (Groszmann and Meurant, personal communication). Degeneration of hybrid progeny is an effective means of isolation (Stebbins 1950) and these observations suggest that *P. edulis* and *P. edulis* f. *flavicarpa* may be so closely related that some inbreeding depression occurs. The later postulate is not supported as no misfit types as described have been observed in several hundred selfed progeny of each of *P. edulis* and *P. edulis* f. *flavicarpa*. It is suggested that these misfit types are not very significant in isolating the two forms, as their frequency is so low. Differences in flowering habit probably constitute the strongest isolating barriers between *P. edulis* and *P. edulis* f. *flavicarpa*.

The F_1 hybrid was generally intermediate to the parental forms in flowering season (table 1) and flowering habit. The flowering season of the F_1 hybrid partially overlapped that of both parents and flowers of the hybrid opened about 9 to 11 a.m. at an intermediate time. These characteristics with the good fertility observed in the F_1 hybrid would enable it to act as a bridging form between the parents.

P. edulis and *P. edulis* f. *flavicarpa* differed in several morphological characters. There was no transgressive segregation exhibited by the six characters in the F_1 generation (table 3, figure 2). The intermediate nature of the hybrid suggested that genes with additive effects are involved in the control of these traits (Lerner 1962). The level of expression of four of the selected characteristics in the hybrid was greater or less than the median rating. Fruit lustre is an exceptional character as it has a similar expression in both the F_1 hybrid and *P. edulis* f. *flavicarpa* suggesting the possible occurrence of dominance. However, no F_2 generation data are available to test these suggestions.

Purple fruit colour was apparently due to a non-dominant factor which would confirm the finding of Nakasone, Hirano and Ito (1967). Fruit colour varies (from 2, 2-3 and 4) among the F_1 vines. Natural hybrids are known and Killip (1938) has mentioned several colour variants of *P. edulis* and apparent intergrades between *P. edulis* and *P. edulis* f. *flavicarpa*. The number of colour classes involved suggests that more than one gene pair may be involved in pigment production. Development of fruit colour in the hybrid may be influenced by segregation of genes controlling this trait, but there is the possibility of variations due to environment and both postulates are not mutually exclusive.

A study of meiosis in *P. edulis*, *P. edulis* f. *flavicarpa* and their hybrid (all $2n = 18$) has indicated more precisely the relationship of the parents (figure 1, table 2). Only regular bivalent behaviour has been observed in *P. edulis* f. *flavicarpa* in Australia (figure 1 (a)) and in Hawaii (Storey 1950). It is probable that this form does not have a hybrid origin as was proposed by Pope (1935). Only normal bivalent behaviour was observed in the F_1 hybrid *P. edulis* x *P. edulis* f. *flavicarpa* (figure 1 (c)), indicating the close homology of the chromosomes of the two forms and the lack of observable structural differences. Storey (1950) has previously suggested a drastic mutation as the mode origin of *P. edulis* f. *flavicarpa* from *P. edulis*. It is more likely that the numerous differences between the two forms are the result of mutation which occurred without drastically altering chromosome homology.

A relatively high chiasma frequency was found in *P. edulis* f. *flavicarpa* (table 2) compared with the other parental form. This is at variance with the postulate of association of low chiasma frequency with adaption to an allogamous system. *P. edulis* f. *flavicarpa* has more highly pigmented and scented flowers than *P. edulis* and self-incompatibility is recorded in clones of *P. edulis* f. *flavicarpa* (Wills, Stephens and Groszmann 1961). The clone of *P. edulis* f. *flavicarpa* involved in this study may not be highly self-incompatible. Its genetic system may be adapted to degrees of recombination vs genetic linkage, different from those of a fully self-incompatible clone. This would indicate why the postulated correlation (Stebbins 1950) of chiasma frequency and breeding system is not apparent between the parental clones. The F_1 hybrid *P. edulis* x *P. edulis* f. *flavicarpa* had a slightly lower chiasma frequency (table 2) than the parental forms. Also, the level of terminalization of chiasmata in *P. edulis* (84.3%) and *P. edulis* f. *flavicarpa* (85.6%) was reduced to 60.7% and 59.6% respectively in the two F_1 plants. The decrease in the level of terminalization in the F_1 hybrid is indicative of a slight lack of homology of the chromosomes of the two parental forms (Darlington 1932).

Speciation is probably presently occurring within *P. edulis*. The hybrid origin suggested for *P. edulis* f. *flavicarpa* (Pope 1935) was not borne out by cytological studies. *P. edulis* and *P. edulis* f. *flavicarpa* differ in several morphological characteristics and the comparative ease of manual crossing between *P. edulis* and its form *flavicarpa* indicate the close genetic relationship of the two forms in spite of their diversity. Differences in flowering season and flowering habit act as barriers to inter-form crossing and ecogeographical barriers also exist. Normal bivalent behaviour was observed in the hybrid *P. edulis* x *P. edulis* f. *flavicarpa* indicating that there were probably no major structural differences differentiating the parental forms. The decreased level of chiasma terminalization in the hybrid only indicated a slight lack of chromosome homology. The presence of barriers to inter-form crossing and the normal meiosis of the hybrid suggest that speciation is presently occurring in *P. edulis* and mutation or minor chromosome interchanges or deletions or combinations of these may be involved.

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