INTRAPOPULATION COMPATIBILITY
IN GONIUM PECITORALE MÜLLER (VOLVOCALES, CHLOROPHYCEAE)

by

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B.Sc., University of British Columbia, 1970

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of
BOTANY

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1974
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Date October 16, 1974
ABSTRACT

Forty clones of *Gonium pectorale* Müller were crossed in all combinations at 20°C, and the resultant zygotes examined to determine the degree of sexual compatibility within a single population. Clones isolated from the same site but in different years were incompatible, indicating a lack of gene flow between them. Two distinct groups were found within 31 clones isolated from a single mud sample, and all the clones in one were incompatible with all those in the other. However, members of both groups were compatible with those in a third group. The existence of at least two complementary pairs of mating types within a single *G. pectorale* population, sensu Stein (1958b), is considered in the context of other sexual compatibility results, and is proposed as a reason for the occasional inability to obtain opposite mating types from a mud sample from which only a few clones have been isolated.
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ACKNOWLEDGEMENTS

I would like to thank Dr. Janet R. Stein for her guidance, patience and encouragement throughout this study.

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I am grateful for the endless support and encouragement given by my husband, Victor.
INTRODUCTION

During the past 20 years, there have been many studies on the compatibility of algal populations, especially on the green flagellates known as the Volvocales. Investigations have dealt both with populations from the same general area and with those widely separated geographically. In these studies (Brooks, 1966; Carefoot, 1966; Coleman, 1959; Goldstein, 1964; Harris and Starr, 1969; Stein, 1958a, b, 1965a, 1966a, b) 10-15 clones were isolated from a single mud or water sample collected from a particular location at a given time. All such clones were considered as belonging to one population. After crossing all isolates in every combination, usually two clones of opposite mating types were kept for further studies on interpopulation compatibility. For some populations opposite mating types were not obtainable within the clones isolated, therefore a single clone was kept for further study. Subsequent crosses frequently demonstrated that the latter clones were compatible with one or several clones from other populations, and therefore were sexual, providing compatible mating types were present. However, it was still unresolved whether populations with one apparent mating type actually consist of (a) only one; (b) two opposite mating types, only one of which appears in the 10-15 clones isolated; or (c) several incompatible mating types of genetically isolated strains.

\[1\] In the colonial Volvocales, a culture started from a single colony (Bold, 1942).
In an attempt to resolve this question, compatibility tests involving many clones from a single population, were carried out. Forty clones of *Gonium pectorale* Müller, isolated from Lemon Cove, California, were crossed at different temperatures. The production of viable zygotes, as indicated by zygote germination, was considered the best criterion for the determination of compatible mating types. Consequently, several techniques were tested to determine the most suitable for inducing zygote germination. The resulting method was used to determine those clones of *G. pectorale* which were compatible.

The life-cycle of *G. pectorale* has been described in detail by Stein (1958b). Karyological studies of this species have been reported by Cave and Pocock (1951) and ultrastructural studies by Lang (1963) and Stein (1965b). The systematics of this species in North America is treated by Pocock (1955).

A colony of *G. pectorale* is a square or rhomboid curved plate composed of 16, sometimes 8, 4 or 2, biflagellated cells interconnected by cytoplasmic strands. The four central cells are surrounded by 12 marginal cells, three on each side, all being enclosed within a gelatinous matrix. Each ovoid to nearly spherical cell contains a single bowl-shaped chloroplast and one or two basal pyrenoids, and is capable of dividing to form a new colony. Each cell also may become a gamete which, upon fusion with a sexually compatible isogamete, forms a zygote. Germination of a smooth-walled zygote or zygospore produces four cells or gones united in a colony, each of which develops into a 16-celled colony. As meiosis occurs during germination, *G. pectorale* is haploid.
in the vegetative condition.

Although homothallic clones have been reported for this species, heterothallic clones are more commonly encountered (Stein, 1965a). The former are those clones which can form fertile zygotes by themselves, and the latter are those which can do so only when mixed with another sexually compatible clone (see Coleman, 1962).

Other terms used in the present study are defined as follows:
- population - "Colonies isolated from one collection site at any one time are considered members of the same population strain and are referred to as either a strain or a population," (Stein, 1958b).
- sexual isolation - a condition in which two populations are incapable of mating and producing viable offspring.
- interpopulation crosses - crosses between clones from two different populations
- intrapopulation crosses - crosses between clones from the same population.
MATERIALS AND METHODS

1. Isolations

Thirty-one clonal cultures were isolated from a dried mud sample according to the technique described by Stein (1958b) (hereafter referred to as the M clones). Isolations were made by Miss Linda Minchin in the summer and fall of 1970 from four different wetted portions of a dried mud sample (Stein Mud 274) collected in August, 1963 from Lemon Cove, California, by Dr. O. Proskauer and stored at room temperature. The 31 clones and their isolation dates are listed in Table I. An additional nine cultures, isolated from dried mud samples collected from the same area but in different years, also were tested for compatibility with the newly isolated clones. The sources and isolation dates of these nine clones (1.6, 13.2, 13.6, 14.1, 14.3, 15.1, 15.3, 57.1 and 57.17 - hereafter referred to as the S clones) are given in Stein (1965a - see Table I).

2. Stocks

Stock cultures were grown in 8 oz jars with small, inverted petri dish lids (65 mm diameter) at a temperature of 20 ± 2°C. They were grown in a 16 hr light - 8 hr dark cycle and at an intensity of 300-350 ft-c. Stocks were transferred every 4-8 weeks. Throughout the study a modified version of Pringsheim's soil-water medium, with a pinch of CaCO₃ added, (Starr, 1964) was used (hereafter referred to as mSWC). The medium was autoclaved at 15 psi for 15 min and allowed to cool for
TABLE I. Isolation dates and size ranges of the M clones.

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24 hr before being used, rather than the usual steaming for one hour on three consecutive days (see Starr, 1964). This modification was adopted to hasten media preparation as no differences were observed in growth rate and mating activity during the preliminary crosses.

3. Crosses

To determine the suitability of the autoclaved medium, observe and recognize the different phases in the life cycle of _G. pectorale_, and establish familiarity with the techniques to be used, several preliminary crosses were tested. All S clones, and numbers 14 to 26 inclusively of the M clones, were chosen for these crosses because of their healthy condition in culture. The crosses were made in sterile watch-glass plates (watch-glass suspended on a glass triangle in a deep petri dish) and spot-plates (triple depression slide in a deep petri dish) (see Hoshaw and Rosowski, 1973; Figure 3-1) and each incubated at 15, 20 or 25°C. Water was added to the petri dishes to prevent excessive evaporation. Concurrently, the remaining clones were transferred every 10-15 days to fresh medium to improve the condition of the cultures.

Upon completion of the tests and development of vigorously-growing cultures, crosses for the main study were prepared by inoculating 1-2 ml from each of two 7-10 day old cultures into a test tube of fresh mSWC medium and incubating at each of the three temperatures specified previously. Sterile pipettes were used for each culture to avoid any possible cross-contamination. Tubes incubated at 15 and 25°C at the
University of British Columbia and all those at the University of Alberta, were kept in test tube racks. Those incubated at 20°C at U.B.C. were suspended by wire hooks on horizontal rods (see Starr, 1973; Figure 11-4). Crosses were kept at the same light intensity and cycle as was used for stock maintenance for the first 2-3 weeks. Subsequently, they were placed at room temperature in complete darkness to permit maturation of any zygotes formed.

All tubes were checked both macro- and microscopically for zygotes one to several weeks after placing in the dark. The presence of an orange ring on the inside of the tube at the air-medium interface and/or of an orange scum on the surface of the medium was indicative of extensive zygote production.

At the University of British Columbia all 40 clones were crossed in every combination and incubated at each of the three test temperatures. During the incubations the culture chambers at 15 and 25°C overheated. As insufficient time was available to repeat the 3200 crosses affected before moving to Edmonton, it was decided to concentrate on the crosses at 20°C. Additionally, since preliminary crosses between the S and M clones showed them to be completely incompatible, the study was restricted further to compatibility solely

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2 As G. pectorale is isogamous, every clone must be crossed with every other clone to determine mating type of each. Additionally, as there are no physical differences, the designation of the mating type, viz. '+' or '-', is purely arbitrary. The mating type designation of each of the S clones follows that given by Stein (1965a), which also was arbitrary.
within the M clones.

At the University of Alberta, the methods used were similar except that the number of crosses possible at any one time was limited, since stocks for crosses were grown only in test tubes. Once the mating type of each clone was determined, clones of opposite mating types were crossed a second time. Additionally, two clones, M17 and M25, which had shown complete incompatibility the first time, were recrossed with all others in every possible combination and, as a test for the tentatively determined mating type of each clone, four of each type were again crossed with all of the other clones of the same mating type. The eight chosen generally were those showing vigorous zygote production in some if not all of the first crosses, and included 'minus' mating types M21, M27, M46 and M53, and 'plus' mating types M4, M10, M18 and M49. All of the above crosses were incubated at 20°C only.

The crosses made at the University of Alberta were allowed to dry partly and sometimes completely after being put into the dark and before checking for zygotes. In dry test tubes zygotes were easily recognized by using a dissecting microscope to scan the walls of the tube. The contents of all tubes also were checked by making a wet mount and examining with a compound microscope. As several of the tubes became contaminated with fungi, a drop of Cotton Blue (Johansen, 1940) was added to each wet mount to differentiate between fungal cells and Gonium zygotes.

All tubes which were found to contain zygotes were stored at room temperature in the dark for subsequent use.
4. Germination Techniques

To obtain sufficient zygotes for testing germination techniques, crosses were made in sterile watch-glasses suspended on glass triangles in deep petri dishes. All were incubated at 20°C and under the same light intensity and regime as used for the crosses described in the preceding section. Two weeks after the crosses were made, the plates, with the lids askew, were put in the dark and left to dry. The watch-glasses were stored at room temperature in individual envelopes.

Various methods have been found successful for inducing zygote germination in the algae. To determine a suitable method for *G. pectorale*, zygotes from six different crosses within the M clones were subjected to each of five different conditions and examined daily for a month for evidence of germination. In all instances germination was found to occur under only one of the five conditions. This method involved placing the zygotes in a sterile watch-glass plate, wetting them with mSWC and then placing them in darkness at 37°C for 48 hr. Water was added to the petri dish to prevent excessive evaporation. After two days at 37°C, the watch-glass plate was placed in a 20°C culture chamber with a 16 hr light - 8 hr dark cycle and subsequent germination was observed within five days.

When zygotes were first subjected to a low temperature (-5°C) for 24 hr before being placed at 20°C, or were placed at 20°C immediately after wetting, no germination was observed. Similarly, attempts to induce germination of zygotes on mSWC supernatant solidified with 1% agar proved unsuccessful, either when exposed to 37°C for 48 hr,
and then 20°C, or when exposed to 20°C only.

Although no attempt was made to quantify the per cent germination, it probably was moderately high since microscopic examination revealed that the majority of the heavy-walled zygotes were empty after five days at 20°C.

To determine the true compatibility of the clones, the viability of the resultant zygotes was determined. During storage in the dark all liquid in the tubes dried up thereby eliminating any vegetative cells. Consequently, rather than transfer the zygotes to sterile watch-glass plates, the successful germination method was applied directly to the zygotes in the test tubes. The presence of colonies in the tubes, as determined with a dissecting microscope, was considered indicative of germination and hence compatibility.
RESULTS

Size ranges for the M clones are presented in Table I. The values for each clone are from measurements made on at least 15 cells and colonies from several cultures which varied in age from 5-20 days. This resulted in a total of 60-80 cells and colonies being measured for every clone.

The data on size ranges for the S clones (Table II) were provided by Dr. J.R. Stein from measurements made prior to this study, as during the present study these clones were destroyed as a result of the equipment malfunctioning.

All of the M clones appear to be *G. pectorale* var. *pectorale* as opposed to var. *praecox* Pocock (Pocock, 1955). This is indicated by: 1) the cell and colony sizes (Table I); 2) the presence of a single pyrenoid; 3) the almost spherical cell shape; 4) the possession of the characteristic bright green colour; 5) the absence of a large central space in the colony (coenobium); and, 6) the absence of precocious daughter colony development. The nine S clones are assumed to be var. *pectorale* also (Stein, personal communication).

The results of the crosses between the S clones are given in Figure 1. Despite the malfunctioning of the 15 and 25°C culture chambers during the main compatibility tests, sufficient data were available from the preliminary crosses that results are reported for 15 and 25°C as well as for 20°C. A positive result is reported only when zygotes were produced in at least two replicate crosses. Clone
TABLE II. Size ranges for the colonies and cells of the S clones provided by Dr. J.R. Stein, (personal communication).

<table>
<thead>
<tr>
<th>Clone No.</th>
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<th>Cell Size (µm)</th>
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FIGURE 1. Intercrossing of the S clones. ▲ - zygotes formed at 15°C; ○ - at 20°C; □ - at 25°C.

FIGURE 2. Germination of zygotes produced by S clone crosses. Clone S1.6 was omitted as it failed to mate with any of the S clones. ▲-zygotes germinated from cross at 15°C; ○ - at 20°C; □ - at 25°C.
S1.6 was incompatible with any of the other eight. There was vigorous zygote production within each of the four populations, viz. S13, S14, S15 and S57, at both 20 and 25°C. At 15°C mating was shown only by population S57.

As indicated in Figure 2, all of the crosses produced viable zygotes.

The results of the crosses between the M clones at 20°C are shown in Figure 3. There were no homothallic clones as none of the self-crosses produced zygotes. Clones M17 and M25 were not compatible with any others in this population. Of the remaining 29 clones, 12 were of the 'minus' mating type and 17 of the 'plus' mating type. There are distinct groupings within each mating type, based on ability to mate. These have been delimited by the heavy lines and each grouping given a letter designation, viz. G, H, J, K and L, to facilitate clarity and eliminate lengthy repetition in the discussion. These letter designations are used in discussing the crosses, i.e. the G x J or H x L crosses, etc.

Clones in group G were compatible with those in group J and, except for clone M2, compatible also with those in group K. The group H clones were compatible with those in both groups K and L, but no zygotes were ever produced from crosses between groups G and L or from crosses between groups H and J.

As shown in Figure 4, considerable variation was observed in the intensity of the mating response. The G x J crosses consistently produced large numbers of zygotes, so much so that the entire surface
FIGURE 3. Intercrossing of the M clones at 20°C. G, H, J, K and L designate groups of clones based on ability to mate within each mating type.  ●—zygotes.
FIGURE 4. Varying intensity of mating response amongst the M clones. G-L: refer to legend for Figure 3. O—extensive mat of zygotes several alyers thick; ■—scattered clumps of zygotes, mat rare; □—small clumps of 10-100 zygotes each, not visible with the naked eye; ▪—very few (10-15) solitary zygotes.
of the medium was covered with a mat several layers thick. Zygote production usually was less vigorous amongst the H x L crosses. Though zygotes were always visible on the surface of the medium, they usually occurred in clumps and rarely formed a mat. In most instances zygotes could not be seen without microscopic examination of test tubes of the crosses involving the K clones though some showed a faint ring at the air-medium interface. Examination with a dissecting microscope revealed small clumps of zygotes (10-100 each) on the walls of the tubes. In other tubes an extensive search produced only 10-15 solitary zygotes. Although the number produced was low, zygote production was consistent.

Despite the variable mating intensity, germination occurred in all zygote-producing crosses (Figure 5), thereby indicating true sexual compatibility. Possible exceptions may be clones M20 and M50; however, crosses involving these two in which no germination was observed, always were contaminated with fungi. As the hyphae completely enveloped the zygotes, they may have had an adverse physical effect on germination.

No zygotes were produced by the crosses between the S clones and the M clones.
FIGURE 5. Germination of zygotes from the M clone crosses. G-L: refer to legend for Figure 3. ■- zygotes from at least two crosses germinated; □- zygotes from only one cross germinated.
DISCUSSION

The degree of sexual compatibility within the S clones reported here, in both inter- and intra-population crosses, is distinctly less than that obtained by Stein (1966b) for the same clones. For comparison, her results are reproduced in Figure 6. She reported all intrapopulation crosses to produce zygotes at 10, 15, 20 and 25°C. In the present study three of the populations, viz. S13, S14 and S15, did not produce zygotes when crossed at 15°C. No crosses were tested at 10°C.

Stein reported five interpopulation crosses, viz. S13.6 x S14.1, S13.6 x S15.3, S14.3 x S15.3, S14.3 x S57.1, and S15.1 x S57.1, that resulted in the production of zygotes, with two of these occurring at two temperatures. In the present study no interpopulation crosses produced zygotes; thus suggesting that the populations are now sexually isolated.

This decrease in sexual compatibility may be a result of maintaining the clones in culture for many years. The S clones had been in culture at least six years when used by Stein, and were at least 12 years old when used in the present study. The period in culture appears to be the only significant variable between the two studies. The culture methods employed in the present study are essentially identical to those used by Stein. The only difference in technique involved the methods of sterilization of the culture medium; however, tests showed there to be no difference in the growth and mating of the clones.
FIGURE 6. Cross results from Stein (1966b) showing interrelationship of clone S1.6 and Minnesota strain 23 (clones 23.1 and 23.5) with the remaining S clones. □- zygotes formed at 10°C; ■- at 15°C; ●- at 20°C; and ○- at 25°C.
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There are two other reports for the Volvocales in which compatibility test results differ between two studies. Brooks (1966) reported several strains of Astrephomene gubernaculifera Pocock (Astrephomeneaceae) and Carefoot (1966) reported two strains of Volvulina steinii Playfair (Volvocaceae) in which mating was observed initially but subsequently could not be induced. With the exception of two of the Astrephomene strains, which apparently lost their capacity for sexual reproduction within several months of isolation, all had been kept in culture for approximately eight years (Stein, 1958a).

The incompatibility of clone S1.6 with the other eight S clones was noted also by Stein (1965a, 1966b). Although she found that it lacked the ability to mate with clones isolated from nearby areas in California, she found it to be compatible with 'minus' clone 23.1 from Minnesota, which in turn was compatible with 'plus' clones S13.6, S14.3 and S15.1. Her data for crosses of Minnesota strain 23 with the California strains (S clones) are included in Figure 6. This interrelationship of Minnesota clone 23.1 with the S clones suggests that clone S1.6 may not be as genetically different from the other S clones as is suggested by the results of the present study. Had an opposite mating type to clone S1.6 been obtained it might have shown this through crosses with some of the S clones. Stein (1966b) points out that "not all interpopulation crosses are reciprocal between the two mating types of two populations" which is evident from the S clone crosses, wherein 'plus' S14.3 x 'minus' S15.3 produced zygotes but 'minus' S14.1 x 'plus' S15.1 did not (Figure 6). Thus non-reciprocal mating may be
a factor in the incompatibility of clone S1.6 with the other S clones.

Non-reciprocal mating between two strains of Pandorina morum Bory (In-BI II and Io-72), led Coleman (1959) to suggest that only one mating type was common to the two pairs of complementary mating types. Since more recent studies (Stein, 1965a, 1966b) indicate that sexual isolation in G. pectorale is not as prevalent as reported previously, it seems more probable that both of Coleman's strains represented a single pair of mating types. The situation concerning clone S1.6 discussed in the preceding paragraph, adds support to the contention that Coleman's non-reciprocal mating more likely was between two strains with the same complementary pair of mating types, than between two strains each representing a different pair of mating types.

Of the 31 M clones only two, M17 and M25, could not be induced to mate with any other clone or with each other. This may be because these two represent a mating type for which the opposite and complementary mating type was not isolated and/or did not survive in culture, as only 31 of the original 55 clones isolated by Minchin survived to produce viable cultures. The incompatibility also could be a result of the temperature at which crosses were incubated, as both these clones grew at a much slower rate than did the others. All crosses were incubated at 20°C, which may differ considerably from the optimum temperature for these two clones. Two additional possibilities exist. Whilst clones M17 and M25 were isolated from rewetted mud, suggesting their sexual origin, the possibility cannot be excluded that they became sterile prior to use in this study, as did two Astrephomene
strains mentioned previously (Brooks, 1966). Alternatively, morphological differences may have been responsible, as clones M17 and M25 were larger than most of the others (Table I). Similar cell size differences as well as several other morphological variations have been reported by Stein (1958b—see Table 6) for 13 G. pectorale strains. However, both the results of that and later studies (Stein, 1965a, 1966b) failed to demonstrate any correlation between morphological characteristics and strain intercompatibility. Subsequent examination of her clones revealed the morphological variation reported to be insignificant (Stein, personal communication).

The occurrence of two distinct mating groups within the remaining 29 M clones suggests that the population consists of two pairs of complementary mating types, members of each pair being incompatible with those of the other. These have been designated +a and -a and +b and -b; groups G and J comprising the former pair and groups H and L the latter. The variation in mating response (Figure 4) gives an indication of the degree of compatibility of the clones within each pair. The G and J clones were highly compatible suggesting a marked

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It is generally assumed that Gonium can survive for extended periods in dried mud samples only as thick-walled zygotes or zygospores. However, the possibility cannot be excluded that this alga is capable of surviving also in the vegetative state, as Trainor and McLean (1964) and McLean (1967) have reported that vegetative cells of Spongiochloris typica Trainor and McLean (Chlorococcales) were viable after one year in a dry state.
similarity in their genetic make-up. The slightly lower numbers of zygotes produced by the majority of L:x H crosses may be indicative of a lesser degree of compatibility between the 17 clones involved, and/or of the culture conditions not being as favorable for mating as they were for the G and J clones.

Considerable variation in intensity of mating reaction was observed also in sexual compatibility studies of *Platydorina caudata* Kofoid (Harris and Starr, 1969). Again, this probably is a reflection of the genetic similarities of the strains, as higher proportions of zygotes always resulted from matings between heterothallic pairs from the same collection site than from matings between pairs from different locations.

Except for the difference in numbers of zygotes generally produced by each of the two pairs of mating types (\(+a\) and \(-a\), \(+b\) and \(-b\)), in this study, no other characteristic was observed that might facilitate their separation. Goldstein (1964) found that of the five intercrossing groups of *Eudorina* strains\(^4\) which he studied, three exhibited a 'clumped' zygote arrangement and two a 'scattered' zygote arrangement. There was almost complete sexual isolation between the 'clumped' and 'scattered' strains, as evidenced by the fact that only two of the 104 intercosses recorded involved strains with differing zygote arrangements. Similarly, Coleman (1959) observed that intercrossing occurred only

\(^4\) Four species and one variety were included; three groups were composed of one species, one of three species, and one of one species and one variety.
between _P. morum_ strains possessing the same type of zygote aggregation, however many strains possessing the same aggregation pattern were sexually incompatible. In _A. gubernalculifera_ intercrossing strains possessed the same chromosome number, there being no intercrossing between strains with different chromosome numbers (Brooks, 1966).

Although the possible existence of multiple pairs of mating types has been shown for some algal species (Coleman, 1959; Starr, 1959; Stein, 1965a), and may be inferred from other published data (Goldman, 1964; Brooks, 1966; Carefoot, 1966), their occurrence within a single population has been studied only once (Brandham and Godward, 1965). Within the 47 clones of _Cosmarium botrytis_ Meneghini tested, they found two distinct pairs of mating types, which they designated + and - and $^+_1$ and $^-_1$, and each pair was incompatible with the other. Their clones were isolated from nine different sites, thus constituting nine different populations according to the definition of 'population' used in the present study. However, within the ten clones isolated from Chobham, Surrey and constituting a single population, two were of the + mating type, two of the - mating type and six of the $^-_1$ mating type. The $^+_1$ mating type may not have been present at all in the Chobham population or may have been missed during the isolation procedure. As only ten clones were isolated the latter appears more probable. They pointed out that if the clones from the Chobham locality were considered alone the six of the $^-_1$ mating type would be assumed sterile. This situation parallels that found in the present study regarding the reproductive status of clones M17 and M25.
Similar also to their findings, the pairs of mating types in the present study were completely incompatible. However, in contrast, each pair was compatible with an intermediate mating type (-^{ab}). This intermediary consists of the three clones of group K (Figure 3) which cross with three of the four +^{a} and all of the +^{b} mating types. It is possible that the exception, clone M2, possesses a fundamental genetic difference which prevents zygote formation. The lack of intermediate + clones may be because they were not isolated and/or did not survive in culture, or because they were inviable (e.g., due to the presence of a sex-linked lethal gene).

The production of variable numbers of zygotes, and very few zygotes in half the crosses involving the K clones, suggests that in general the K clones have a very low compatibility with the G and H clones. Thus the K clones may have been produced by intercrosses between the two pairs of complementary mating types. If so, they would be very susceptible to slight variations in culture conditions, hence the varied results. Alternatively, they may act as the intermediary stage towards the development of a separate interbreeding group of clones arising from either complementary pair, e.g., H and L from G and J or vice versa. However, this would be very unlikely unless the K clones consistently produced large numbers of zygotes in crosses with either the G or H clones, thereby ensuring the production of a vigorous population in which subsequent mutations could lead to the establishment of a new and isolated interbreeding population. Instead, the results support the contention that the K clones originated as a result
of previous interbreeding between the two presently genetically isolated pairs of mating types.

The genetic similarity of the two pairs of mating types ($+^a$ and $-^a$ and $+^b$ and $-^b$), indicated by their common compatibility with the K clones, suggests that they arose from the same stock, either one from the other or both from a common parent. It is possible that both occurred in different (but possibly overlapping) niches in the pond thus enabling both to co-exist. Support for this contention is the differing degrees of compatibility observed, in that the niche for the $+^a$ and $-^a$ clones may be more closely approximated by the laboratory conditions than it may be for the $+^b$ and $-^b$ clones. This then could explain the differing numbers of zygotes consistently produced by the crosses.

The five additional populations comprising the S clones were included in the compatibility tests with the M clones because they originated from the same or nearby areas. Three of the five, S13, S14 and S57, were collected from the same pond but in different years, viz. 1951, 1955 and 1956 respectively. The M clones also were collected from this pond, but in 1963. Consequently, these four populations may be considered as a 'local population', or community of potentially interbreeding individuals at a given locality (Mayr, 1970). However, according to Stein (1958b), the difference in collection dates would negate the four being considered as belonging to the same population, and therefore they would be outside the scope of a strict 'intrapopulation' study.
Although Stein (1966b) found that the three strains, S13, S14 and S57, were compatible to a certain degree, (Figure 6, modified from Stein, 1966), Coleman (1959) reported in *P. morum* two sexually isolated groups existing amongst the seven clones or strains isolated on four different occasions from a pond designated 'Bloomfield I'. This pond was sampled repeatedly to determine if mating types were of constant occurrence and Coleman found that two of the collections contained a different pair of mating types. Two collections were made from each of five other ponds and in every instance the two pairs of mating types were incompatible, suggesting to her that, "... the coexistence of two sexually isolated *Pandorina* populations in a single pond is not an uncommon occurrence."

The results of crosses of *G. pectorale* have already demonstrated the inability of the S clones to interbreed and this incompatibility is extended to crosses with the M clones. The S clones, except for clone S1.6, originally were interfertile suggesting that they all belong to one pair of mating types. This pair then, may be incompatible with the pairs of mating types present in the M clones. Alternatively, the population in the pond represented by the M clones may be sexually isolated from the populations which existed there seven or more years previously, simply as a result of evolutionary change.

The fact that multiple pairs of mating types may exist within single populations suggests a reason for the occasional inability to isolate opposite mating types from some populations. It is conceivable that
a more extensive study might show the existence of an unlimited number of reproductively isolated units within a single population.
SUMMARY

The present study has reaffirmed the existence of multiple, reproductively isolated breeding units in Gonium pectorale Müller, previously demonstrated by Stein (1965a), within populations from diverse geographical locations. In contrast to her results, two incompatible pairs of complementary mating types are reported within a single population, represented by 31 clones isolated from a dried mud sample.
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