UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Mating-type differentiation and mate selection in the homothallic Chlamydomonas monoica

van den Ende, H.; VanWinkle-Swift, K.P.

DOI
10.1007/BF00357164

Publication date
1994

Published in
Current Genetics

Link to publication

Citation for published version (APA):
van den Ende, H., & VanWinkle-Swift, K. P. (1994). Mating-type differentiation and mate selection in the homothallic Chlamydomonas monoica. *Current Genetics*, *25*, 209-216. https://doi.org/10.1007/BF00357164

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:11 Mar 2023

Curr Genet (1994) 25:209–216

Current Genetics

© Springer-Verlag 1994

Mating-type differentiation and mate selection in the homothallic *Chlamydomonas monoica*

Herman van den Ende [1], **Karen P. VanWinkle-Swift** [2]

[1] Department of Molecular Cell Biology, University of Amsterdam, Kruislaan 318, NL-1098 SM Amsterdam, The Netherlands
[2] Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, USA

Received: 7 July 1993 / Accepted: 23 August 1993

Abstract. By using combinations of phenotypically-distinct – but sexually-compatible – mutant strains of *C. monoica* (*zym-1*, *zym-27*, and *nit-2*) and assaying for zygote genotypes in genetically-mixed mating populations (where gametes of the two parental homothallic strains were present at similar frequencies), we have found that matings occur preferentially between cells of the same genotype. Additional support for an hypothesis of non-random mate selection was provided by using an easily-selectable genetic marker (*sup-1*) to verify the frequent occurrence of matings between cells of identical genotype in populations where the selectable genotype was present at very low relative frequency (10^{-2} or 10^{-3}) in a mixed mating population. Direct evidence for non-random mate selection was obtained by presenting nitrogen-starved cells with limiting nitrate to synchronize gametic differentiation in wild-type strains. Under these conditions, the four, eight, or 16 mitotic daughters released from the same mother sporangium often immediately established mating pairs within the group. Thus successive mitotic divisions of a single mother cell yielded progeny of opposite expressed mating-type.

Key words: *Chlamydomonas monoica* – Homothallism – Mating-type differentiation – Mate selection

Introduction

The unicellular alga *C. monoica* is homothallic: sexually-compatible mating-types appear within a population derived from a single haploid cell (Strehlow 1929; VanWinkle-Swift and Bauer 1982; Van den Ende et al. 1992). Mating interactions resemble those of the heterothallic species *C. eugametos* (Musgrave et al. 1985; Van den Ende et al. 1988). Compatible gametes form vis-a-vis pairs with flagellar paralysis of one partner allowing the other to direct the movement of the pair. Although gametes remain walled, cell fusion is accomplished by partial anterior wall lysis and extension of the activated mating structures which establish a cytoplasmic connection (Shi and VanWinkle-Swift, in preparation). Uniparental inheritance of chloroplast genes also suggests that the gametes of *C. monoica* are equivalent to the opposite mating-types (mt^+ and mt^-) of heterothallic species (Van Winkle-Swift and Aubert 1983). However, nothing is known about the cellular and genetic mechanisms that give rise to opposite mating-types within a clonal population, i.e. from a single progenitor cell.

There are two necessary conditions for zygote formation in *C. monoica*: nitrogen starvation and the ability to divide (Van den Ende et al. 1992). Nitrogen-starved cells that are unable to divide do not mate. Thus, simply transferring vegetative cells to nitrogen-free medium (a technique routinely used in other *Chlamydomonas* species; Sager and Granick 1954; Trainor 1958) has not proven useful for gamete induction in *C. monoica*. Instead, media best described as nitrogen-*limiting* (VanWinkle-Swift and Bauer 1982; Van den Ende 1992) promote the differentiation of vegetative cells into mating-competent gametes. Upon transfer to nitrogen-limiting media, cells continue to undergo a few rounds of cell division before mating ensues. It is likely that upon attainment of mating competence, the cells are nitrogen-starved and incapable of further division.

Previous work on zygote formation in this species (Van den Ende et al. 1992) suggests that successful mating is not dependent upon random collisions but that some form of mate selection may be occurring. In particular, increasing the population density during gametogenesis did not result in an increase in zygote yields. The correlation between the potential for continued cell division and increased mating efficiency led to the hypothesis that mitotic daughters selectively establish mating pairs, perhaps initiating effective contact while still within the confines of the mother cell wall – a process we will henceforth refer to as *sib-mating*.

This hypothesis predicts that within a mixed population of genetically-distinct but sexually-compatible (and

Correspondence to: K. P. VanWinkle-Swift

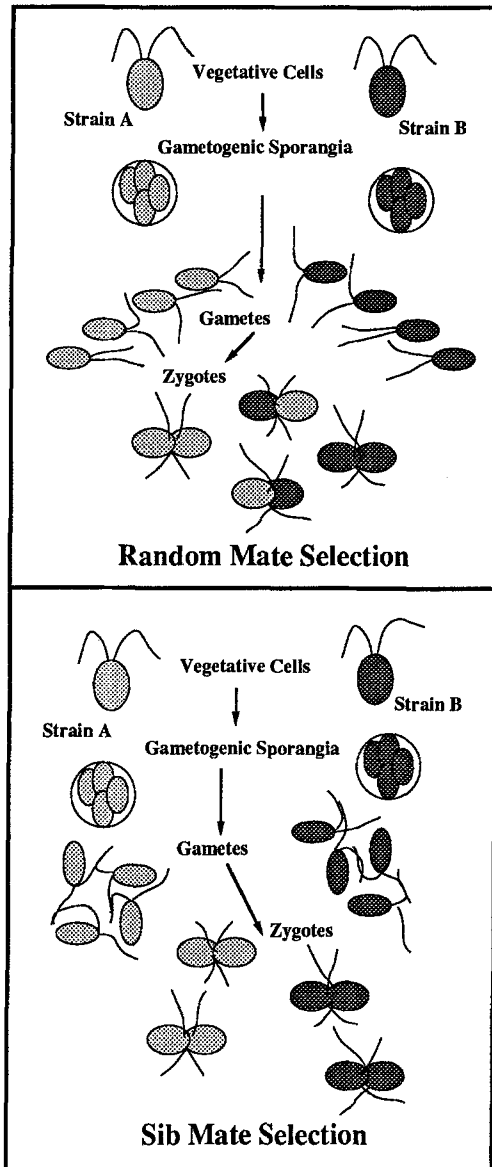

Fig. 1. Alternative hypotheses for mate selection in genetically-mixed homothallic mating populations. According to the hypothesis of random mate selection, gametes derived from one parental strain are as likely to mate with members of a second strain as with members of their own strain. This assumes that gametes of each genotype are equally abundant in the population and that the ratios of opposite mating-types produced by the two strains are the same. In contrast, the hypothesis of sib-mate selection predicts that matings between members of different strains will be rare. Instead, most gametes will pair with siblings released from the same mother cell sporangium. This assumes that each sporangium produces gametes of both mating-types and in equal numbers

homothallic) strains, mating within strains should be more common than mating between strains. In contrast, assuming an equal frequency of gametes of the two strains, a model invoking random mate selection would predict a 1:1 ratio of homozygous zygotes (from selfing) and heterozygous zygotes (from crossing). Figure 1 illustrates the two alternative hypotheses for mate selection in *C. monoica*. We describe below the application of a variety of phenotypically-distinctive mutant strains to test the hypothesis of non-random mate selection in genetically-mixed but homothallic populations. As predicted, the frequency of heterozygotes produced by matings between the parental genotypes is always less than expected based on a model of random mate selection. Furthermore, we show that a given parental genotype can efficiently produce homozygous zygotes even when present at extremely low relative abundance in a genetically-mixed population. By shifting cells from nitrogen-free medium to nitrogen-limiting medium we have now been able to synchronize the gametogenic divisions and verify – by direct microscopic observation – extensive sib-mate selection.

Materials and methods

Strains. The *C. monoica* strains used in this study include the wild-type strains *WT15c* (VanWinkle-Swift and Burrascano 1983) and UTEX 220 (from the University of Texas Algal Culture Collection), a nitrate non-utilizing auxotroph (*nit-2*; unpublished), two recessive lethal zygote-maturation mutants, *zym-1* (VanWinkle-Swift and Bauer 1982) and *zym-27* (Parmelee and VanWinkle-Swift 1983), a germination-defective mating-type-limited strain *mtl-1-2* (carrying a more stringent mutant allele at the *mtl-1* locus described by VanWinkle-Swift and Hahn 1986), and a double mutant carrying the *mtl-1-2* lethal allele and a recessive suppressor, *sup-1*, of this lethality (VanWinkle-Swift et al., submitted).

Media and culture conditions. Mutant and wild-type strains are routinely maintained on agar-solidified nitrate-containing Bold's Basal medium (BM; Bishcoff and Bold 1963) or on ammonium-containing High Salt medium (HS; Suoeka 1960). Asynchronous mixed mating populations containing two genetically- and phenotypically-distinct strains were established in liquid nitrate-limited LPN medium (VanWinkle-Swift and Bauer 1982). Strains are maintained at 20–23 °C under continuous cool white fluorescent illumination (30–60 μE m^{-2} sec^{-1}).

Mixed mating populations. Mixed mating populations for the *zym-1/zym-27* or *nit-2/WT15c* combinations were established as follows: each parental strain was suspended in LPN medium to a final cell density of 1×10^6 cells ml^{-1}; 1-ml mixed mating populations were created by mixing 200, 300, 400, 500, 600, 700 or 800 µl of one parental strain with 800, 700, 600, 500, 400, 300, or 200 µl of the other strain. Because the efficiency of gametogenesis varies from strain to strain, or from one experiment to another, a starting ratio of 1:1 vegetative cells of each strain will not necessarily produce a 1:1 ratio of parental gametes. Gamete genotype frequencies can be deduced from the resultant zygote genotype frequencies (see legend to Table 2). Only data from mating populations that were later deduced to have involved approximately-equal gamete genotype frequencies are reported here. Mixed mating populations for the *mtl-1-2/mtl-1-2 sup-1* combination of parental strains were established by suspending vegetative cells of each parental strain in LPN to a final density of 1×10^6 cells ml^{-1}. The *mtl-1-2 sup-1* suspension was then diluted 100 fold. To create the 100:1 biased mating culture, 500 µl of the undiluted *mtl-1-2* culture was mixed with 500 µl of the diluted *mtl-1-2 sup-1* culture. To create the 1 000:1 biased mating culture 900 µl of the undiluted *mtl-1-2* culture was mixed with 100 µl of the diluted *mtl-1-2 sup-1* culture. In analyzing the data from these experiments (see Fig. 3 and Table 2), we assumed that the efficiencies of gametogenesis for the two strains were similar. (Zygote yields obtained in control self-matings of the individual parental cultures indicated mating efficiencies of 13% for *mtl-1-2* and 11% for the *mtl-1-2 sup-1*). Analysis of these mixed mating populations also rests on assumptions concerning the viability (germination efficiency) of the *mtl-1-2* and *mtl-1-2 sup-1* homozygotes. Zygote densities were determined by a hemacytometer count for self-matings of the *mtl-1-2* and *mtl-1-2 sup-1* strains and known numbers of zygotes

were plated, matured, and induced to germinate following standard procedures (VanWinkle-Swift and Bauer 1982). From 60000 *mtl-1-2* homozygotes plated, six colonies were recovered (germination efficiency $=10^{-4}$). From 263 *mtl-1-2 sup-1* homozygotes plated, 245 colonies were recovered (germination efficiency=0.93). For simplicity, in making predictions (see Fig. 3 and Table 2) we have considered the germination efficiency of *mtl-1-2 sup-1* to be equivalent to 1.0)

Assessing zygote genotypes and phenotypes. To determine the phenotypes (and genotypes) of zygotes produced in mixed mating populations containing the *zym-1* and *zym-27* strains, aliquots from 7-day-old LPN cultures were examined by phase-contrast light microscopy to determine zygote morphology (see text and Fig. 2 for further details). From 500 to 1 000 zygotes were scored for each *zym-1/zym-27* mating population analyzed. For mating populations containing the *nit-2* and *WT15c* strains, aliquots from 7-day-old LPN cultures were plated on BM agar, incubated in the dark for 3–5 days and then induced to germinate as described previously (VanWinkle-Swift and Bauer 1982). The latter procedure results in a color distinction between *nit-2* (yellow) and wild-type (green) segregants from meiotic germination of zygotes (see text for further details). For each *nit-2/WT15c* mating population, 100–200 zygote clones were scored for color after 5–7 days post-germination growth. Viable zygotes produced in mixed mating populations containing the *mtl-1-2* and *mtl-1-2 sup-1* strains may be derived from rare germination of *mtl-1-2* homozygotes (frequency 10^{-4}), rare germination of *mtl-1-2 sup-1* heterozygotes (frequency 10^{-4}), or from normal germination of *mtl-1-2 sup-1* homozygotes. The zygotes derived from rare germination of *mtl-1-2* homozygotes are easily identified by "self-mating" each viable zygote clone and retesting the second generation of zygotes for germination. To distinguish between *mtl-1-2 sup-1* homozygotes and the rare germination of zygotes heterozygous for *sup-1*, 50 viable zygote clones derived from these populations were subcloned. Twenty-five subclones from each zygote clone were then self-mated and tested for germination. All viable zygote clones previously shown not to be *mtl-1-2* homozygotes, proved to be homozygous for both the *mtl-1-2* and *sup-1* alleles, i.e., no segregation of the unsuppressed *mtl-1-2* germination defect was observed.

Synchronization of gametogenesis. Details of the synchronization procedure are provided in the text. Briefly, vegetative cells are first transferred to nitrogen-free medium for 15–24 h in darkness (or under an alternating light/dark regime) to deplete intracellular nitrogen reserves. The starved cells are then shifted to a nitrate-limiting medium for 18–24 h under continuous illumination. At the end of this period the culture is comprised primarily of sporangia (containing four, eight, or 16 mitotic daughters) which hatch synchronously to release mating-competent gametes.

Photographic procedures. Gametogenic sporangia and mating cells were observed at 400 × magnification, and zygotes at 1 000 ×, using a Zeiss Axioplan phase-contrast microscope equipped with an Hitachi color video camera (Model KP-C501). Images were viewed on an Hitachi color monitor (Model CT1365VM) and recorded on TDK-Extra High Grade VHS tape using an Hitachi Video Tape Recorder (Model VT-F430A). Images were captured as arrested single frames from the video tape, and were converted to a monochrome image using a Sony Video Graphic printer (Model UP-811).

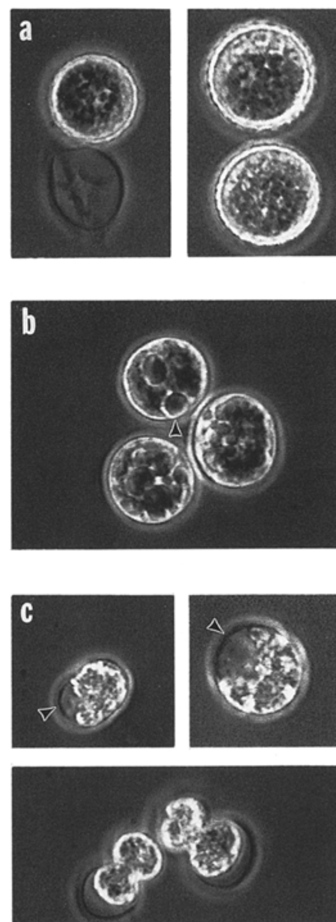

Fig. 2a–c. Wild-type and mutant zygote phenotypes. **a** Wild-type zygospores (homozygous or heterozygous) have a distinctive reticulate surface. A primary zygote wall (lower part of left panel) is released as maturation continues. Zygotes produced by matings between *zym-1* and *zym-27* gametes have the wild-type phenotype shown here. **b** Zygotes produced by self-mating of *zym-1* gametes tend to associate in clumps, have a smooth non-reticulate surface, and show no evidence of the primary zygote wall. Large lipid globules (*arrow*) are also clearly visible in most *zym-1* homozygotes. **c** Zygotes produced by self-mating of *zym-27* gametes acquire a very granular appearance often with evidence of extensive plasmolysis (*arrows*, upper panels). Alternatively, *zym-27* homozygotes are found constricted into dumb-bell shapes upon attempting to release the primary zygote wall (lower panel)

Results

In mixed mating populations where two genotypes are present at similar frequencies, matings occur more frequently within strains (genotypes) than between strains

To test the hypothesis that mate selection is non-random in genetically-mixed homothallic populations, we first used two mutant strains carrying zygote-specific lethal markers that alter zygote morphology in distinctive ways. Figure 2a–c compares the morphologies of wild-type, *zym-1*, and *zym-27* homozygous zygotes. The *zym-1* and *zym-27* alleles are complementary and matings between the two strains yield wild-type heterozygous zygotes (Van Winkle-Swift and Burrascano 1983; VanWinkle-Swift 1989). As shown in Table 1a, the frequency of heterozygotes produced by matings between the two strains was always less than that predicted on the basis of random mate selection. Instead, matings occurred preferentially between gametes of the same genotype.

Similarly, mixed mating populations were prepared using the nitrate non-utilizing *nit-2* and the wildtype *WT15c* strains. After allowing for mating and zygote for-

Table 1. Zygote genotypes derived from mixed mating populations comprised of two homothallic parental genotypes in nearly equal abundance

(1) *zym-1*, *zym-27* mixed mating populations

Gamete genotype frequencies[a]		Zygote genotype frequencies[b]					
zym-1	*zym-27*	Homozygous				Heterozygous	
		zym-1/zym-1		*zym-27/zym-27*		*zym-1/+ zym-27/+*	
		Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
0.52	0.48	0.50	0.27	0.46	0.23	0.05	0.50
0.56	0.44	0.49	0.31	0.36	0.19	0.14	0.49
0.43	0.57	0.36	0.18	0.50	0.32	0.14	0.49
0.55	0.45	0.42	0.30	0.32	0.20	0.25	0.50
0.46	0.55	0.34	0.21	0.45	0.30	0.21	0.51

(2) *nit-2*, *WT15c* mixed mating populations

Gamete genotype frequencies[a]		Zygote genotype frequencies[b]					
WT15c	*nit-2*	Homozygous				Heterozygous	
		WT15c		*nit-2*		*nit-2/+*	
		Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
0.53	0.47	0.41	0.28	0.48	0.22	0.11	0.50
0.56	0.44	0.49	0.31	0.38	0.19	0.13	0.50
0.31	0.69	0.31	0.10	0.68	0.48	0.00	0.42
0.55	0.45	0.50	0.30	0.42	0.20	0.07	0.50
0.63	0.37	0.58	0.40	0.33	0.14	0.09	0.47

[a] Relative gamete frequencies are calculated as, for example, [2(no. of *zym-1* homozygotes)+(no. of heterozygotes)]÷2 (total no. of zygotes)=original freq. of *zym-1* gametes

[b] Predictions for homozygote and heterozygote frequencies are based on the assumption of random mate selection and are calculated, for example, as follows:
pred. freq. of *zym1* homozygotes=(freq. *zym-1* gametes)^2
pred. freq. of *zym-27* homozygotes=(freq. *zym-27* gametes)^2
pred. freq. of heterozygotes=2 (freq. *zym-1* gamete) (freq. *zym-27* gamete),
or 1-(freq. of *zym-1* homozygotes+freq. of *zym-27* homozygotes).
The observed zygote genotype frequencies are simply the number of zygotes showing the *zym-1*, *zym-27* or wild-type (heterozygous) zygote phenotype divided by the total number of zygotes observed

mation, the zygotes were germinated on nitrate (BM) medium. Under these conditions, zygotes formed by matings between wild-type gametes produce green colonies, while those formed from matings between *nit-2* gametes produce yellow colonies. Matings between the two strains will produce heterozygotes that segregate *nit-2* and *nit-2*$^+$ alleles and so produce green/yellow sectored zygote clones. As shown in Table 1b, matings between the *WT15c* and *nit-2* gametes occurred less frequently than expected if mate selection were random.

In a genetically mixed homothallic mating population, even an extremely rare genotype mates preferentially with cells of the same genotype

To increase the stringency of our tests for non-random mate selection, a selectable genetic marker was needed to detect very rare mating events between cells of an uncommon genotype within a background of matings between cells of a more abundant genotype (or crosses between the rare and abundant genotypes). Mixed mating populations were established using cells of a germination defective strain, *mtl-1-2*, present in a 100- or 1 000-fold excess over those of a strain carrying – in addition to the lethal *mtl-1-2* allele – a suppressor of the germination defect, *sup-1*. All matings in this population thus produce zygotes homozygous for *mtl-1-2*. Because the suppressor is recessive, matings between the rare *mtl-1-2 sup-1* cells and the abundant *mtl-1-2* cells do not produce a viable zygote. Only matings between the rare *mtl-1-2 sup-1* cells themselves produce zygotes that germinate efficiently. However, the *mtl-1-2* lethal allele shows a low level of leakiness such that one in 10 000 zygotes homozygous for *mtl-1-2* is viable. Thus, the zygote genotype must be verified by subcloning (see Materials and methods.)

Figure 3 shows the ways in which viable zygotes can be produced in these mixed populations according to either a random mating or sib-mating hypothesis for mate selection. The hypothesis of random mate selection predicts that many of the viable zygote clones recovered will be derived from rare germination of the more common

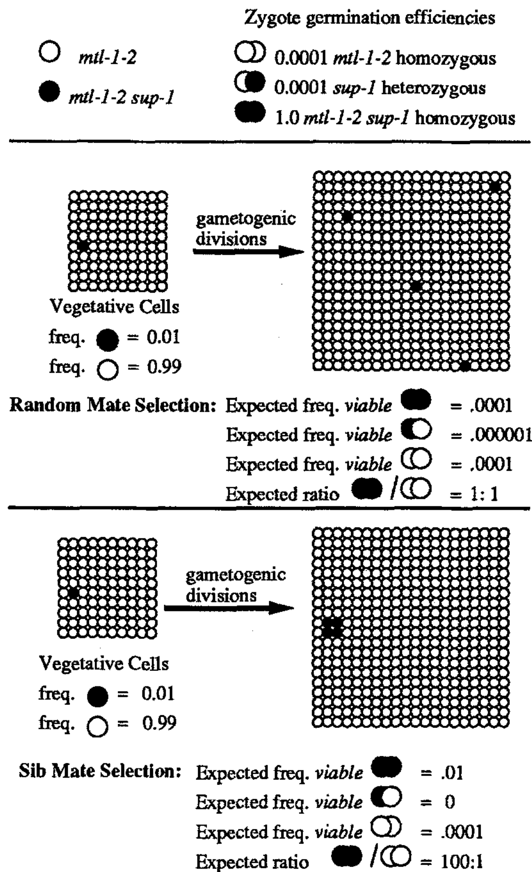

Fig. 3. Predictions for recovery of zygotes homozygous for a rare genotype from a highly-biased mixed homothallic mating population. At ratio of 100:1, viability from *mtl-1-2* leakiness (10^{-4}) is equal to that of rare random *mtl-1-2 sup-1* × *mtl-1-2 sup-1* encounters ($10^{-2} \times 10^{-2}$). Thus, 50% of the viable zygotes recovered from the population would be expected to be homozygous for *sup-1*, while the others would prove upon re-testing to be pure for the *mtl-1-2* germination defect. If the bias were increased to 1000:1 (see Table 2), only 1% of the viable zygotes are expected to be homozygous for *sup-1*. In contrast, if encounters between *mtl-1-2 sup-1* gametes are in fact achieved by sib-mating, then the expected frequency of viable zygotes homozygous for *sup-1* is equal to the frequency of the *mtl-1-2 sup-1* gametes (10^{-2}) since encounters will occur with 100% efficiency as long as the original *mtl-1-2 sup-2* vegetative cells produce gametogenic sporangia. Because this expected frequency is 100-fold higher than the germination efficiencies of *mtl-1-2* homozygotes (which will make up the vast majority of the zygote population), approximately 99% of the viable zygote population is expected to be homozygous for *sup-1*. If the bias is increased to 1000:1 (see Table 2), the frequency of *sup-1* homozygotes will drop to 10^{-3}; however, this is still 10-fold higher than the germination efficiency (10^{-4}) of the *mtl-1-2* homozygotes. See Table 2 for the actual results obtained

mtl-1-2 homozygotes. In contrast, efficient sib-mating would promote the recovery of the *mtl-1-2 sup-1* homozygous zygospores. The proportion of viable zygotes homozygous for *sup-1* (Table 2) is completely incompatible with an hypothesis of random mate selection. The rare *mtl-1-2 sup-1* gametes found genetically-identical mating partners with apparent ease – an observation that could be explained if matings occurred between mitotic daughters from a single gametogenic sporangium. The few viable zygote clones recovered that were not homozygous for *mtl-1-2 sup-1* proved upon further analysis (see Materials and methods) to be homozygous for *mtl-1-2* and arose as a consequence of the low level of leakiness associated with this lethal mutation. As expected, based on the recessive nature of the *sup-1* allele (VanWinkle-Swift et al., submitted), matings between the rare *mtl-1-2 sup-1* gametes and the abundant *mtl-1-2* gametes (if such matings occurred at all) were not a source of viable zygotes.

Table 2. Recovery of zygotes homozygous for a rare genotype in highly-biased mixed mating populations

Parental genotypes ratios *mtl-1-2*:*mtl-1-2 sup-1*		No. of viable zygotes homozygous for *sup-1*		
		Observed (vs total viable)	Predicted[a]	
			RM[b]	SM[c]
100:1	A[d]	432 (433)	216.5	429
	B[d]	248 (249)	124.5	247
1000:1	A	140 (176)	2	158
		109 (114)	1	103
	B	354 (398)	4	358
		235 (236)	2	212

[a] See Fig. 3 for methods for predicting the frequencies of viable zygotes that will be homozygous for *mtl-1-2* versus *mtl-1-2 sup-1*
[b] RM, prediction based on assumption of random mating
[c] SM, predictions based on assumption of sib-mate selection
[d] A and B are separate experiments using independently-isolated alleles at the *sup-1* locus that similarly suppress the germination defect of *mtl-1-2* strains

Direct microscopic observation of sib mate selection

These genetic observations emphasized the need to synchronize gametogenic divisions in order to observe directly whether the hypothesized sib-mate selection was occurring. The routine procedure for genetic analysis in *C. monoica* utilizes a low nitrate medium that promotes asynchronous gamete formation 24–48 h after the transfer of parental vegetative cells to the nitrogen-limiting environment. We report here two alternative protocols that improve synchronization of gamete formation in *C. monoica:*

(1) Cells grown in nitrate-limited continuous culture (Van den Ende 1992) are subjected to a 15 h dark period in nitrogen-free Bold's Basal medium. Nitrate is then added to a final concentration of 0.1 µmoles per 10^5 cells. After 18 h of continuous illumination, the population undergoes synchronous mitotic divisions giving rise to four-, eight- and 16-celled sponrangia. A detailed description of this protocol will be presented elsewhere (Van den Ende, submitted). As the daughter cells are released by partial autolysis of the sporangial wall they agglutinate immediately by their flagella. (Mating pairs fuse within 1 h to produce the quadriflagellate zygote which then matures into the thick-walled zygospore.)

(2) Vegetative cells maintained on HS agar for a minimum of 14 days are suspended in nitrogen-free Bold's Basal medium and are placed in an alternating 15 h light/

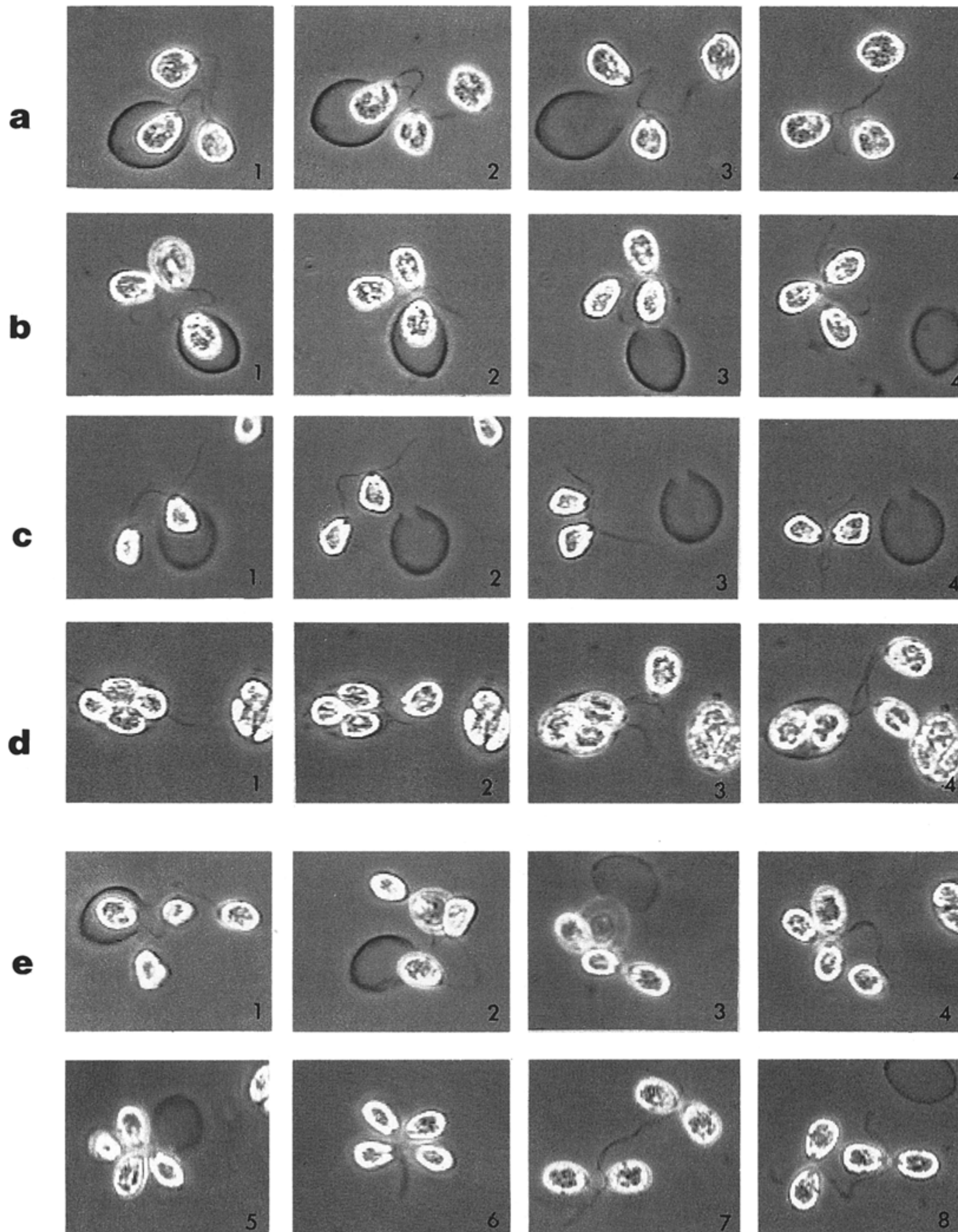

Fig. 4a–e. Sib-mating in *C. monoica*. Panels are numbered to indicate temporal progression of the gametic interactions. **a** and **b** the first cell released from the mother sporangium has escaped without interacting with its sibs (data not shown). The remaining three siblings show typical gametic flagellar agglutination which assists in the escape of the fourth gamete from the sporangial wall. These gametes can ultimately yield only one mating pair. **c** The first two cells released from the sporangial wall have escaped (data not shown). The third gamete exits and is shown here assisting the fourth gamete to escape the mother cell wall. These two siblings then establish a vis-a-vis mating pair. **d** The first gamete released assists in the release of the second gamete and proceeds toward pair formation via extensive flagellar agglutination with its sibling. This pair ultimately moved away from the sporangium. The third gamete escaped leaving the fourth entrapped within the sporangial wall (data not shown). **e** As hatching progresses, all four gametes (including one still within the sporangial wall) are seen to be interacting via their flagella. After the fourth gamete is pulled free of the wall by its siblings, flagellar agglutination reactions proceed to the establishment to two sib-mating pairs

9 h dark cycle for 24–72 h. At the dark-to-light transition, cells are recovered by centrifugation, resuspended in LPN medium (0.6 m*M* nitrate; VanWinkle-Swift and Bauer 1982) to a final density of approximately 5×10^6 cells ml^{-1}, and placed under continuous illumination. Synchronous mitotic divisions to yield four- or eight-celled sponrangia occur 18–21 h later. Upon release of the daughter cells from the mother cell sporangium, immediate flagellar agglutination among siblings often occurs.

Figure 4 shows several sequences of images from video recordings of synchronized gamete release and sib-mate selection in the UTEX 220 wild-type strain of *C. monoica*. Synchronization of *WT15c* or of other genetically-marked strains has yet to be accomplished. Differences in the physiology of genetically-distinct strains may require adjustments to the precise level of nitrate, or the conditions of illumination prior to and/or during gametogenesis.

Discussion

In mixed mating populations containing genetically-distinct strains, the frequency of mating within strains is often higher than expected if mate selection were random; conversely, matings between strains often occur less frequently than expected. Although the increased frequency of intra-strain mating observed in many populations may be largely a consequence of sib-mating, such inbreeding can also be enhanced by differences in the timing of attainment of mating competence by the two strains. If one strain aquires agglutinability prior to the other, gametes of the precocious strain are likely to have chosen their partners before the other strain attains competence. The problem is exacerbated by the rapid loss of agglutinability in *C. monoica* soon after gamete formation (Van den Ende 1992). Gametes must find partners quickly.

The most reasonable explanation for the apparent ease with which very rare genotypes within a population find mating partners of the same genotype is to assume that mating-competent gametes can be produced prior to release of mitotic daughters from the mother sporangial wall, and that these gametes establish effective flagellar agglutination prior to or at the time of sporangial wall lysis. Crowding of gametes within the sporangium makes it unlikely that effective flagellar agglutination could occur there unless cell division positions opposite mating-types such that their flagella can make contact. However, immediate agglutination upon sporangial wall lysis – including flagellar interactions between the first gametes released and their sibs still within the sporangium – has been observed (Fig. 4). In cases where mating competence is not fully established at the time of sporangial wall lysis (e.g., in the absence of light), the subsequent selection of mating partners will occur essentially at random, i.e., gametes will be more likely to select non-sib partners – which may or may not be derived from the same genetic background.

Sib-mating may be common among immotile homothallic organisms. For example, in *Saccharomyces cerevisiae* the proper orientation of two budding sister cells can result in the formation of two zygotes (Hicks and Herskowitz 1976; Nasmyth 1982). However, it is remarkable that mating between sibs is not avoided in a motile haploid organism. The nature of cellular division in *C. monoica* results in the transient retention of fully-differentiated gametes of opposite mating-type within the mother sporangium and promotes non-random, sib-mate selection (via flagellar agglutination) upon lysis of the sporangial wall. Although sib-mating may occur at variable frequencies depending upon environmental conditions that affect the synchronization of gametic divisions and gamete release, the fact that it occurs at all is of tremendous survival value to the species. Although homothallism per se reduces the potential difficulty in finding a mate of opposite sex, sib-mating reduces even further the distances that need to be travelled to find a partner and accentuates the inbreeding associated with homothallism in this species.

This mating strategy – which is essentially independent of cell density – differs from that used by heterothallic species such as *C. eugametos* where gamete encounters are strongly dependent upon the density of opposite mating-type cells in the population (Lewin 1956; Tomson et al. 1986). The potential for sib-mate selection may be particularly advantageous for a species colonizing an empty niche or one whose numbers have been greatly reduced. Nevertheless, the question remains as to why cells which propagate very efficiently vegetatively use sex at all. Many procaryotes and lower eucaryotes (including *Chlamydomonas*) survive under conditions where vegetative propagation is not possible by sporulation and entrance into dormancy. For *Chlamydomonas*, then, the more relevant question may be why it uses sex to produce the spore (see Goodenough 1985 for discussion). One might imagine that diploidy per se is of selective value for survival under environmental stress where chromosomal damage and mutation may occur at increased frequency. Diploidy not only allows masking of mutations arising in the spore but, in species where diploidy is achieved by sex and subsequently terminated by meiosis, the potential also exists for recombinational or meiotic repair regardless of how inbred the original mating strategy may have been.

Acknowledgements. This work was supported, in part, by NSF grant 9106008 to K.V.W.S. Technical assistance was provided by Ms. Amy Parks-Ritter and Julie Flynn. We thank Drs. A Gibor, R. F. Hoekstra, and A. Musgrave for helpful conversations.

References

Bischoff HW, Bold HC (1963) Phycological studies. IV. Some soil algae from enchanted rock and related algal species. Univ Texas Publ no. 6818

Goodenough UW (1985) An essay on the origins and evolution of eukaryotic sex. In: Halvorson HO, Monroy A (eds) The origin and evolution of sex. Alan R. Liss, New York, pp 123–140

Hicks JB, Herskowitz I (1976) Interconversion of yeast mating types. I. Direct observations of the action of the homothallism (HO) gene. Genetics 83: 245–258

Lewin RA (1956) Control of sexual activity in *Chlamydomonas* by light. J Gen Microbiol 15: 170–185

Musgrave A, de Wildt P, Schuring F, Crabbendam K, Van den Ende H (1985) Sexual agglutination in *Chlamydomonas eugametos* before and after cell fusion. Planta 166: 234–243

Nasmyth KA (1982) Molecular genetics of yeast mating type. Annu Rev Genet 16: 439–500

Parmelee J, VanWinkle-Swift KP (1983) The ultrastrucutre of zygote maturation in *Chlamydomonas* J Cell Biol 97: 31 a

Sager R, Granick S (1954) Nutritional control of sexuality in *Chlamydomonas reinhardi*. J Gen Physiol 37: 729–742

Strehlow KZ (1929) Uber die Sexualitat einiger Volvocales. Z Bot 21: 625–692

Sueoka N (1960) Mitotic replication of deoxyribonucleic acid in *Chlamydomonas reinhardi*. Proc Natl Acad Sci USA 46: 83–91

Tomson AM, Demets R, Sigon CAM, Stegwee D, Van den Ende H (1986) Cellular interactions during the mating process in *Chlamydomonas eugametos*. Plant Physiol 81: 522–526

Trainor FR (1958) Control of sexuality in *Chlamydomonas chlamydogama*. Am J Bot 45: 621–626

Van den Ende H, Klis FM, Musrave A, Stegwee D (1988) Sexual agglutination in *Chlamydomonas eugametos*. In: Chapman GP, Ainsworth CC, Chatham CK (eds) Eukaryote cell recognition: concepts and model systems. Cambridge University Press, Cambridge, pp 92–104

Van den Ende H, Van den Briel ML, Lingeman R, Van der Gulik P, Munnik T (1992) Zygote formation in the homothallic green alga *Chlamydomonas monoica* Strehlow. Planta 188: 551–558

Van Winkle-Swift KP (1989) Developmental genetics of *Chlamydomonas*. J Phycol 25 (suppl): 12

VanWinkle-Swift KP, Aubert B (1983) Uniparental inheritance in a homothallic alga. Nature 303: 167–169

VanWinkle-Swift KP, Bauer JC (1982) Self-sterile and maturation-defective mutants of the homothallic alga *Chlamydomonas monoica* (Chlorophyceae). J Phycol 18: 312–317

VanWinkle-Swift KP, Burrasacano CG (1983) Complementation and preliminary linkage analysis of zygote maturation mutants of the homothallic alga, *Chlamydomonas monoica*. Genetics 103: 429–445

VanWinkle-Swift KP, Hahn J-H (1986) The search for mating-type-limited genes in the homothallic alga *Chlamydomonas monoica*. Genetics 113: 601–619

Communicated by R. W. Lee