UvA-DARE (Digital Academic Repository)

Random amplified polymorphic DNA markers (RAPD's) as tools for gene mapping in *Chlamydomonas eugametos* (chlorophyta)

Haring, M.A.; Schuring, F.; Urbanus, J.H.M.; Musgrave, A.; van den Ende, H.

DOI

[10.1111/j.0022-3646.1996.01043.x](https://doi.org/10.1111/j.0022-3646.1996.01043.x)

Publication date

1996

Published in

Journal of phycology

[Link to publication](#)

Citation for published version (APA):

Haring, M. A., Schuring, F., Urbanus, J. H. M., Musgrave, A., & van den Ende, H. (1996). Random amplified polymorphic DNA markers (RAPD's) as tools for gene mapping in *Chlamydomonas eugametos* (chlorophyta). *Journal of phycology*, 32, 1043-1048. <https://doi.org/10.1111/j.0022-3646.1996.01043.x>

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.

RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS (RAPDs) AS TOOLS FOR GENE MAPPING IN *CHLAMYDOMONAS EUGAMETOS* (CHLOROPHYTA)¹

Michel A. Haring,² Frank Schuring, Jos Urbanus,
Alan Musgrave, Herman van den Ende

Institute of Molecular Cell Biology, BioCentrum Amsterdam, University of Amsterdam,
Kruislaan 318, 1098 SM, Amsterdam, The Netherlands

and

Patrick J. Ferris

Department of Biology, Washington University, Box 1229, One Brookings Drive, St. Louis, Missouri 63130

ABSTRACT

Studying the sexual behavior of the heterothallic green alga *Chlamydomonas eugametos* Moewus has revealed genetic differences among the few available strains: different O-methylated sugar patterns on plasma membrane glycoproteins (*oms A/B* locus) and light-(in)dependent sexual agglutinin activation (*lsa* locus). However, due to the lack of a reliable linkage map, genes controlling these traits are not accessible by map-based cloning. Here we present a partial linkage map for *Chlamydomonas eugametos* based on random amplified polymorphic DNA (RAPD) markers and one restriction fragment-length polymorphism (RFLP) marker. Most of these RAPDs (80%) represent unique DNA sequences, which implies that they can be used as starting points for chromosome walking. RAPDs linked to the *lsa* locus, the mating type locus (*mt*), and the *oms A/B* locus were identified by bulk segregant analysis.

Key index words: bulk segregant analysis; *Chlamydomonas eugametos*; Chlorophyta; isogenic lines; linkage map; RAPDs; RFLPs

Over the years we have studied sexual processes in the heterothallic, biflagellate, unicellular green alga *Chlamydomonas eugametos* Moewus in great detail (Musgrave 1993, van den Ende 1994). Analysis of the cellular and biochemical parameters determining successful mating revealed genetic differences between the two haploid *C. eugametos* strains studied, UTEX 9 mating type plus (*mt*⁺) and UTEX 10 mating type minus (*mt*⁻). The first genetic difference described was the occurrence of different O-methylated sugar groups on many glycoproteins of the plasma membrane (Gerwig et al. 1984, Homan et al. 1987, Schuring et al. 1987). Only one set of O-methylated sugars occurs in each strain, 4-O-methyl xylose, 2-O-methyl arabinose, and 3-O-methyl galactose (detectable with monoclonal 44.42, group A) in UTEX 9 and 6-O-methyl mannose and 3-O-methyl glucose (detectable with monoclonal 44.2, group B)

in UTEX 10 (Homan et al. 1987). These traits behave genetically as alleles of one gene (Schuring et al. 1987), which we here name *oms A/B* (O-methylated sugar group A or B). The occurrence of O-methylated sugar group A or B is inherited independent of the mating type. So far, no function has been assigned to their occurrence.

The second genetic difference we studied was the light-dependent change in flagellar adhesiveness. Light-sensitive strains (e.g. UTEX 9) lose their agglutinability in the dark because their flagellar agglutinins are inactivated. When illuminated, they rapidly become agglutinable again. This process is restricted to agglutinins on the flagellar membrane, whereas those on the plasma membrane of the cell body are unaffected (Kooijman et al. 1988). Agglutinins of light-insensitive strains (e.g. UTEX 10) do not require light for activation; they are continuously active. We found that the property of light-sensitivity is inherited in a Mendelian fashion and that the difference appears to involve a single locus, which we name *lsa* (light-sensitive agglutination). *lsa* is not linked to the mating type locus (Kooijman et al. 1986) nor to the *oms A/B* locus.

Molecular cloning of the genes responsible for these two genetic differences would help us to elucidate their function. Because we lack information about the genes and their products, we resorted to chromosome walking, a technique that was successfully applied to cloning mating type associated genes in *Chlamydomonas reinhardtii* (Ferris and Goodenough 1994). For this purpose, we constructed a linkage map of DNA and phenotypic markers for the strains UTEX 9 and 10. We searched for restriction fragment-length polymorphisms (RFLPs) using random complementary DNA (cDNA) clones and four different restriction enzyme digests of UTEX 9 and 10 DNA. We tested more than 50 different cDNA probes and found only one polymorphism. We also tested the interfertile strains UTEX 96 and 97 and SAG 23/91 and 24/91 (isolates from Göttingen, Germany). In 50% of the cases, we found RFLPs with UTEX 9 and 10. However, as UTEX 96 and 97 give less than 5% viable offspring in crosses with

¹ Received 11 April 1996. Accepted 26 August 1996.

² Author for reprint requests.

TABLE 1. Sequence of decamer primers that generated RAPDs between *C. eugametos* UTEX 9 and 10. Approximate size of the generated bands is in kilobasepairs (kb).

Primer	Nucleotide sequence (5' to 3')	Polymorphic markers (kb)
A18	A G G T G A C C G T	1.3
B10	C T G C T G G G A C	1.6
B13	T T C C C C C G C T	0.85
C01	C C G G C C G T C A	1.45
C16	G C G A T T G G G G	1.95
C18	G C C G A A C C G G	1.45
C19	A G T C T G G C G T	1.8
V14	A G A T C C C G C C	0.7 and 1.0
W07	C T G G A C G T C A	2.7
X01	C T G G G C A C G A	0.2 and 1.95
Z10	C C G A C A A C C	1.45
Z14	T C G G A G G T T C	1.3 and 3.0

UTEX 9 or 10 (Gowans 1963, Cain 1979, Lee and Lemieux 1990), these strains cannot be used for genetic mapping. Furthermore, the SAG 23/91 and 24/91 strains differ in more than one gene with regard to the *lsa* phenotype (F. Schuring, results not shown), making mapping of this interesting locus very complicated. Considering our interest in the *lsa* locus, we tried to find DNA polymorphisms between UTEX 9 and 10 by means of the random amplified polymorphic DNA (RAPD) technique (Welsh and McClelland 1990, Williams et al. 1990). This method was previously used to make linkage maps of higher plants (Giese et al. 1994) and insects (Hunt and Page 1995) and to detect polymorphisms in inbred strains of mice (Woodward et al. 1992).

MATERIALS AND METHODS

Chlamydomonas eugametos strains UTEX 9 (mt⁺) and UTEX 10 (mt⁻) and *Chlamydomonas moewusii* strains UTEX 96 (mt⁺) and UTEX 97 (mt⁻) were obtained from the Culture Collection of Algae, University of Texas at Austin. The *C. eugametos* strains SAG23/91 (mt⁺) and SAG24/91 (mt⁻) were isolated in 1990 from mud from a fish pond in Göttingen and were kindly provided by Dr. U. G. Schlösser (Göttingen, Germany). Cells were grown on agar-containing M1 medium (Wiese 1965) kept at 20°C in a 12:12 h LD regime, with an irradiance of 30 $\mu\text{mol photons}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Gamete suspensions were obtained by flooding 2–4-week-old cultures with distilled water.

Zygote Formation, Maturation, and Germination

Crosses were initiated by mixing equal amounts of mt⁺ and mt⁻ gamete suspensions of the selected parental cultures. The agglutinating cells were illuminated for 2 h at 23°C to form a high percentage of so-called vis-à-vis pairs. Zygote development took place after pipetting 0.25 mL of the mating suspension onto agar plates (Oxoid, Unipath Ltd., Basingstoke Hampshire, England) containing M1 medium. The plates were placed in the light, at 24°C for 24–48 h depending on the rate of development. The plates were then wrapped in aluminium foil and placed in the dark at 24°C for 7–12 days. Zygotes and nonfused vegetative cells were subsequently scraped from the agar surface with a sterile razor blade and transferred in a streak to a fresh culture plate. Single mature zygotes were positioned in a row at some distance from the surrounding cells. A Zeiss inversion microscope and a glass needle (drawn out from a Pasteur pipette) were used for manipulating the zygotes on the agar surface. Plates were then incubated in the light at 24°C, inducing the diploid zygotes

FIG. 1. RAPDs generated on DNA from the *C. eugametos* strains UTEX 10 and SAG23/91 and their progeny (tetrad a, b, c, and d) with the primers C10 and C17. Genetic markers: bands A–F.

to go through meiosis and germination within 24–48 h. The four haploid cells representing the meiotic products of one zygote were separated at some distance from each other in a row perpendicular to that of the isolated zygotes. Colonies arising from the isolated cells were transferred to fresh agar plates containing M1 medium. The resulting F₁ clones were maintained under the same conditions as their parents.

Screening Phenotypic Traits

Mating type. Gamete suspensions of the progeny clones were tested for their ability to mate with the parental mt⁺ and mt⁻ strains (Schuring et al. 1987).

Light sensitivity. Cells were flooded with distilled water just before the start of the dark period and placed directly in the dark (dark gametes). The agglutination activity of these dark gametes was tested by mixing them with light-insensitive gametes of the opposite mating type in the dark and in the light. Strains able to agglutinate in the dark and light are referred to as light-insensitive whereas gametes that only agglutinate in the light are called light-sensitive (Kooijman et al. 1986).

O-methylated sugars. The O-methylated sugar group expressed by each F₁ clone was detected by using an indirect immunofluorescence test (Homan et al. 1987). Mab 44.42 and 44.2 are monoclonal antibodies that recognize *C. eugametos* strain-specific glycoprotein epitopes containing, respectively, 4-O-methyl xylose, 2-O-methyl arabinose, and 3-O-methyl galactose (group A sugars) and 6-O-methyl mannose and 3-O-methyl glucose (group B sugars). Gametes were fixed in a mixture of glutaraldehyde and formaldehyde (final concentration of 0.33 and 1%) for 5 min, washed 3 times in phosphate-buffered saline (PBS), pH 7.2, and incubated for 30–60 min with the appropriate Mab supernatant diluted 15 times in PBS. Nonbound antibodies were removed by washing twice in PBS. Bound antibodies were labeled with 100 times diluted goat-anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) (Tago Inc., Burlingame, California). Cells were examined under an Olympus epifluorescence microscope (fitted with DM-500, BP-490, and EY-455 filters) for the presence of A or B sugar group-carrying glycoproteins on their flagellar surfaces.

DNA Extraction

Cells were centrifuged at 13,000 rpm for 1 min in an Eppendorf centrifuge, after which 100–150 μL of cell pellet was mixed with

FIG. 2. Hybridization of gel-purified RAPD fragments with blots of *C. eugametos* genomic DNA UTEX 9 and 10 digested with *Pst*I (P) and *Hind*III (H). Numbers below the lanes indicate the RAPD fragment used as probe. When one primer generated two RAPDs the size of the used fragment is indicated. Molecular weight markers are presented in kilobasepairs (kb).

an equal volume of glass beads (diameter 490–900 μ m) and 500 μ L extraction buffer (20 mM Tris-HCl, pH 8.0, containing 25 mM EDTA, 0.1% SDS, 0.1% Triton X-100, 15 mM DTT, 100 μ g·mL⁻¹ proteinase K). The mixture was vortexed for 1 min and then incubated at 45° C for 20 min and vortexed several times during this incubation. Sixty microliters 3 M NaAc and 500 μ L phenol was added, vortexed for 1 min, and centrifuged for 5 min at 13,000 rpm. The aqueous phase from each tube was transferred to a new Eppendorf tube. Five hundred microliters phenol/chloroform (1:1) was added, the mixture was vortexed for 10 s, and the samples were centrifuged for 5 min at 13,000 rpm. The aqueous phase was transferred to a sterile tube. The nucleic acids were precipitated with 1 mL of 96% ethanol for 5 min at room temperature, centrifuged at 13,000 rpm, and washed twice with 70% ethanol. The samples were vacuum-dried and resuspended in 200 μ L sterile ddH₂O containing 20 μ g·mL⁻¹ RNase H (Boehringer, Mannheim) and incubated at 37° C for 30 min and the DNA concentration and quality was estimated by agarose gel electrophoresis. DNA was stored at -20° C.

Primers

Oligonucleotide primers (10-mers) were purchased from Operon Technologies (Alameda, California). The sequence of the primers and the size of the RAPD fragment they generated are listed in Table 1.

Amplification Conditions

PCR were performed in volumes of 25 μ L containing 3 μ L template DNA (50–100 ng) of the appropriate dilution, 3 μ L

primer (15–18 ng), 0.25 μ L mix of dNTPs (10 mM each of dATP, dCTP, dGTP, and dTTP), 2.5 μ L of 10 $\times$ Super Taq buffer, 0.25 units of Super Taq enzyme (HT Biotechnology Ltd., Cambridge, England) and 16.2 μ L sterile ddH₂O. The mixture was overlaid with a drop of mineral oil, and the amplifications were performed in a Pharmacia Gene ATAQ Controller. The PCR program starts with 4 min at 95° C followed by 36 cycles of 1 min denaturation at 94° C, 1 min annealing at 35° C, and a 2-min extension step at 72° C, using the fastest available ramp time. Amplification products were analyzed by electrophoresis in 1.2% agarose gels containing 0.5 μ g·mL⁻¹ ethidium bromide.

Southern Blots

Individual RAPD marker bands were excised from agarose gel, and the DNA was purified using the GeneClean II kit (Bio 101 Inc., La Jolla, California). Isolated fragments were labeled with α -³²P dATP by random primer labeling and used as a probe on a DNA blot. Genomic DNA was cut with *Pst*I and *Hind*III separated on a 0.7% agarose gel. DNA was transferred onto Qiabran nylon blotting membrane (Qiagen, Hilden, Germany) or Hybond N nylon membrane (Amersham). Hybridization with radioactive probes was done according to the instructions of the manufacturer.

RESULTS

We first optimized the protocol for generating RAPDs with regard to the quantity of DNA (50–100 ng), primer concentration (15 ng), annealing

FIG. 3. Segregation of RAPDs and their hybridizing DNA fragments in two complete tetrads of a cross between *C. eugametos* UTEX 9 and 10. Panel A shows the segregation of a RAPD generated with primer C1 (arrow and white circles). The hybridization of this 1.45-kb fragment with the DNA gel blot is shown in panel C. The band co-segregating with the C1 RAPD is marked by a white circle. Panel B shows the segregation of a RAPD generated with primer C16 (arrow). The hybridization of this 1.95-kb band to the DNA gel blot is shown in panel D. The DNA used for gel blot analysis was digested with *Pst*I. Phenotypes of the parents and the progeny: + or - indicates mating type, A or B means *omsA* or B phenotype.

temperature (35° C), number of cycles (35), and, most importantly, the source of Taq-polymerase. Probably due to the high G + C content of *Chlamydomonas* DNA, the working range of the annealing temperature proved to be very narrow, but under standardized conditions RAPDs were reproducibly generated. To establish whether a polymorphism between the tested strains was due to a genetic or an artifactual difference we tested a complete tetrad for segregation of the RAPD (Fig. 1). It is clear that artifactual differences between DNA samples occurred, but the genetic markers (bands A–F) were easily identified because they segregated 2:2 in the tetrad. Using this tetrad control, we detected 15 RAPDs between UTEX 9 and 10 by testing 138 primers (12 primers that did not yield bands were not included). For comparison, we found 85 RAPDs between UTEX 10 and SAG23/91 from 100 primers tested.

Because our major aim was to use the RAPDs as unique DNA markers from which to start chromosome walks, we tested whether the amplified DNA fragments contained unique or repetitive sequences. Individual fragments were gel-purified and used for hybridizations with *Pst*I and *Hind*III digests of ge-

nomic *C. eugametos* UTEX 9 and 10 DNA (Fig. 2). Of 10 probes tested, 8 recognized unique sequences, whereas 2 probes identified repetitive sequences in both strains (probes V14 (0.7) and X1, Fig. 2). Although a RAPD fragment was identified as being a difference between the two parental strains, it did not necessarily generate an RFLP in the subsequent DNA gel blot analysis. Only 5 of the 10 probes tested gave an RFLP with the two enzymes tested (e.g. probe V14 (1.0) and W7, Fig. 2). Some of the RAPD probes (W7, C01, and C16) gave hybridization patterns with extra bands in one strain only. We then compared the results of the RAPD marker segregation with the segregation pattern of the hybridizing fragment in the DNA gel blot analysis (Fig. 3). RAPD C1 is an example of a probe that recognized two DNA fragments (panel C), one of which was clearly segregating with the RAPD pattern (panel A). This fragment was linked to the plus mating type locus and could be used to start a walk into this region. The RAPD fragment C16 hybridized to a single DNA fragment that was present only in strains exhibiting the *omsA* phenotype. The data from Figures 2 and 3 confirmed that RAPD fragments can be used as probes for the start of a chromosome

FIG. 4. Bulk segregant analysis of *lsa*⁺ and *lsa*⁻ pools with primer Z14. The left panel illustrates RAPD patterns obtained with primer Z14 using the parental DNAs and DNAs from two complete tetrads. The right panel shows the equivalent patterns from the *lsa* pools. The arrow indicates the Z14(3.0) band present only in the *lsa*⁺ pool. The star marks the spot where the Z14 (1.3) band was expected to appear in the pools. This band is visible in samples 20a, b and 24b, d.

walk, because they recognized unique DNA fragments and followed the same segregation pattern as the RAPDs in DNA hybridization studies.

To find markers linked to the *lsa* locus, we decided to use bulk segregant analysis (Tanksley et al. 1995). In this approach, DNA from phenotypically identical progeny was pooled. These strains will share the chromosome region that contains the locus of interest but are heterogeneous in the rest of their genome. Only DNA polymorphisms in the region of interest will be detected. DNA from selected progeny (*lsa*⁺ or *lsa*⁻) of 10 tetrads was pooled and used for a PCR with RAPD primers. The effectiveness of this approach using primer Z14 was evident when the 3.0-kb band only appeared in the *lsa*⁺ pool indicating linkage to this locus (Fig. 4). Subsequent analysis of single progeny from 44 tetrads mapped the Z14 (3.0) band at a distance of 18 centimorgan from the *lsa* locus (Fig. 5). The second RAPD band, Z14 (1.3) was barely visible in either of the *lsa* pools but was tightly linked to the mating type minus locus when *mt*⁺ and *mt*⁻ pools were analyzed (data not shown). With *omsA* and *omsB* pools, we identified RAPDs C16 (1.95) and W7 (2.7) as markers linked to the *omsA* locus (see genetic map Fig. 5). It therefore seems likely that one can rapidly identify RAPDs linked to a trait by bulk segregant analysis.

Construction of a RAPD-linkage map of C. eugametos. The segregation of 15 RAPD markers, 3 phenotypic markers (mating type, *omsA/B*, *lsa*), and 1

FIG. 5. Linkage map of *C. eugametos* UTEX 9 based on 15 RAPD markers, 3 phenotypic markers, and 1 RFLP marker. *mt* = mating type, *lsa* = light-sensitive agglutination, *omsA* = *O*-methylated sugar group A. Distances are indicated in cM.

RFLP marker (VH-PTP13 cDNA, Haring et al. 1995) was determined in 44 complete tetrads from a cross between UTEX 9 and 10. These tetrads were classified as PD (parental ditype), NPD (nonparental ditype), or T (tetatype) (Harris 1989). The number of PD, NPD, and T tetrads obtained indicated whether the loci in question were linked or not. Linkage was determined by pairwise comparisons of all markers using the formula $(4\text{NPD} + 2\text{T}) / [4(\text{PD} + \text{NPD} + \text{T})] \times 100$. This resulted in seven linkage groups (Fig. 5). Most of the RAPD markers (5) were closely linked to the mating type locus (*mt*, linkage group VI), as could be expected for this highly polymorphic region (Ferris and Goodenough 1994). RAPD marker C16 co-segregated with the *omsA* phenotype, for in 66 tetrads we found complete linkage to the presence of the A-group of *O*-methylated sugars. This tight linkage means that the C16 fragment is ideal for starting a chromosome walk toward the *omsA* gene.

DISCUSSION

RAPD markers were previously used for mapping complex higher plant genomes (Giese et al. 1994). This is the first demonstration of how useful RAPDs in *Chlamydomonas* can be for the construction of linkage maps. The technique is simple and has the advantage that only a small amount of DNA (less than 100 ng) is required for the generation of a RAPD

pattern. The DNA quality does not have to be extremely high. More importantly, the generation of DNA markers is independent of DNA probes. This advantage is clearly illustrated by the fact that we were unable to produce DNA markers using cDNA probes, but we readily detected polymorphisms using the RAPD technique. Apparently, this technique is very suitable for finding DNA polymorphisms between near-isogenic or inbred lines (Woodward et al. 1992). Nevertheless, one has to be careful with nonspecific differences in the RAPD patterns between DNA samples. Genetic markers can be easily distinguished from nonspecific differences by analyzing complete tetrads. The segregation of a RAPD marker should always be in agreement with the expected Mendelian 2:2 segregation.

Our inability to find a workable number of RFLPs between UTEX 9 and 10 and the relatively few RAPDs found illustrates that these laboratory strains are near-isogenic. The other isolates differ greatly from UTEX 9 and 10, for we found 6–10 times more polymorphisms with the RAPD technique. A cross between one of these polymorphic strains and UTEX 9 or 10 could be used to make a high-density linkage map. So far, we have identified seven linkage groups for *C. eugametos*. Because most of the linkage groups consist of only one marker, it remains to be seen whether they are independent linkage groups.

Our main purpose was to find DNA markers suitable for starting a chromosome walk toward the *lsa* or the *oms A/B* locus. The fact that most of the RAPD fragments seem to occur as single-copy DNA sequences qualifies them for this goal. The RAPD C16 fragment that is linked to the *oms A/B* locus is a good example. We have used it to start a chromosome walk in *C. eugametos* UTEX 9 and UTEX 10 and discovered that C16 is only present in UTEX 9 because this strain contains a DNA stretch of approximately 52 kb that is not present in UTEX 10 (F. Schuring, P. Ferris, and M. Haring, unpubl.). Not only have we demonstrated that the RAPD technique is a powerful tool to generate DNA markers in near-isogenic lines, the bulk segregant analysis using RAPDs appears to be a very promising technique to find markers linked to a trait of interest. For each primer, only four PCR reactions will have to be carried out: two parental DNA samples and the two DNA pools. The classical approach would require at least the analysis of 10 tetrads (40 DNA samples) for the detection of linkage to a certain phenotype.

This work was partly supported by the Foundation for Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research (NWO). P.J.F. was supported by an NWO visitor's grant.

Cain, J. R. 1979. Survival and mating behaviour of progeny and germination of zygotes from intra- and interspecific crosses

- of *Chlamydomonas eugametos* and *C. moewusii* (Chlorophyceae, Volvocales). *Phycologia* 18:24–9.
- Ferris, P. J. & Goodenough, U. W. 1994. The mating-type locus of *Chlamydomonas reinhardtii* contains highly rearranged DNA sequences. *Cell* 76:1135–45.
- Gerwig, G. J., Kamerling, J. P., Vliegenhart, J. F. G., Homan, W. L., van Egmond, P. & van den Ende, H. 1984. Characteristic differences in monosaccharide composition of glycoconjugates from opposite mating types of *Chlamydomonas eugametos*. *Carbohydr. Res.* 127:245–51.
- Giese, H., Holm-Jensen, A. G., Mathiasen, H., Kjaer B., Rasmussen, S. K., Bay, H. & Jensen, J. 1994. Distribution of RAPD markers on a linkage map of barley. *Hereditas* 120: 267–73.
- Gowans, C. S. 1963. The conspecificity of *Chlamydomonas eugametos* and *Chlamydomonas moewusii*: an experimental approach. *Phycologia* 3:37–44.
- Haring, M. A., Siderius, M., Jonak, C., Hirt, H., Walton, K. M. & Musgrave, A. 1995. Tyrosine phosphatase signalling in a lower plant: cell-cycle and oxidative stress-regulated expression of the *Chlamydomonas eugametos* VH-PTP13 gene. *Plant J.* 7:981–8.
- Harris, E. H. 1989. *The Chlamydomonas Sourcebook*. Academic Press, San Diego, California, 780 pp.
- Homan, W. L., Kalshoven, H. W., Kolk, A. H. J., Musgrave, A., Schuring, F. & van den Ende, H. 1987. Monoclonal antibodies to surface glycoconjugates in *Chlamydomonas eugametos* recognize strain-specific O-methyl sugars. *Planta (Berl.)* 170: 328–35.
- Hunt, G. J. & Page, E. G., Jr. 1995. Linkage map of the honey bee, *Apis mellifera*, based on RAPD markers. *Genetics* 139: 1371–82.
- Kooijman, R., de Wildt, P., Homan, W. L., Musgrave, A. & van den Ende, H. 1988. Light affects flagellar agglutinability in *Chlamydomonas eugametos* by modification of the agglutinin molecules. *Plant Physiol.* 86:216–23.
- Kooijman, R., Elzenga, T. J. M., de Wildt, P., Musgrave, A., Schuring, F. & van den Ende, H. 1986. Light dependence of sexual agglutinability in *Chlamydomonas eugametos*. *Planta (Berl.)* 169:370–8.
- Lee, R. W. & Lemieux, C. 1990. Loss of hybrid lethality during backcross programs involving *Chlamydomonas eugametos* and *Chlamydomonas moewusii* (Chlorophyceae). *J. Phycol.* 26:376–80.
- Musgrave, A. 1993. Mating in *Chlamydomonas*. *Progr. Phycol. Res.* 9:193–237.
- Schuring, F., Smeenk, J. W., Homan, W. L., Musgrave, A. & van den Ende, H. 1987. Occurrence of O-methylated sugars in surface glycoconjugates in *Chlamydomonas eugametos*. *Planta (Berl.)* 170:322–7.
- Tanksley, S. D., Ganai, M. W. & Martin, G. B. 1995. Chromosome landing: a paradigm for map-based gene cloning in plants with large genomes. *Trends Gen.* 14:937–43.
- van den Ende, H. 1994. Vegetative and gametic development in the green alga *Chlamydomonas*. *Adv. Bot. Res.* 20:125–61.
- Welsh, J. & McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acid Res.* 18:7213–8.
- Wiese, L. 1965. On sexual agglutination and mating-type substances (gamones) in isogamous heterothallic chlamydomonads. I. Evidence of the identity of the gamones with the surface components responsible for sexual flagellar contact. *J. Phycol.* 1:46–54.
- Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. & Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acid Res.* 18:6531–5.
- Woodward, S. R., Sudweeks, J. & Teuscher, C. 1992. Random sequence oligonucleotide primers detect polymorphic DNA products which segregate in inbred strains of mice. *Mamm. Genome* 3:73–8.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.