12. L. Felicetti, B. Colombo, C. Baglioni, *Biochim. Biophys. Acta* **119**, 120 (1966).
13. W. Wilbrandt, *Trans. Faraday Soc.* **33**, 956 (1937).
14. B. Lindemann and H. Passow, *Pflügers Arch. Ges. Physiol.* **271**, 369 (1960); *ibid.*, p. 488; *ibid.*, p. 497.
15. L. B. Kirschner, *Arch. Biochem. Biophys.* **106**, 57 (1964).
16. R. L. Post, C. R. Merritt, C. R. Kinsolving, C. D. Albright, *J. Biol. Chem.* **235**, 1796 (1960).
17. B. Roelofsen, H. Baadenhuysen, L. L. M. Van Deenen, *Nature* **212**, 1379 (1966).
18. H. Noll, F. O. Wettstein, T. Staehelin, *ibid.* **198**, 632 (1963); B. Hardesty, R. Miller, R. Schweet, *Proc. Nat. Acad. Sci. U.S.* **50**, 924 (1963); H. Lamfrom and P. M. Knopf, *J. Mol. Biol.* **9**, 558 (1964).
19. R. W. Hendler, *Nature* **193**, 821 (1962); *ibid.* **207**, 1053 (1965); L. D. Moore, F. J. Kocun, W. W. Umbreit, *Science* **154**, 1350 (1966).
20. E. A. Smuckler and E. P. Benditt, *Biochemistry* **4**, 671 (1965); T. E. Webb, G. Blobel, V. R. Potter, *Cancer Res.* **26**, 253 (1966).
21. A. Miller and A. B. Maunsbach, *Science* **151**, 1000 (1966).
22. A. Kornberg, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955), vol. 2, p. 497.
23. W. D. Love and G. E. Burch, *J. Lab. Clin. Med.* **41**, 351 (1953).
24. M.H. is a fellow of the Anna Fuller Fund, on leave from the Institute of Microbial Chemistry, Tokyo, Japan.

12 April 1967

Apomixis: Seasonal and Population Differences in a Grass

Abstract. *Changes in incidence of apomictic and sexual embryo sacs were detected in* Dichanthium aristatum *in an experimental population at six stations covering 27 degrees of south latitude, and during the flowering season in a wild population. Differences were associated with photoperiods prevailing during development of inflorescences. Response to length of day was quantitative, differing in the two strains.*

A cytological survey of inflorescences of *Dichanthium aristatum* grown under field conditions reveals striking changes in the incidence of apomixis during the flowering season. These changes are strongly associated with differences in photoperiod during development of inflorescences. Apomixis in *Dichanthium* is of the aposporous type (*1*), in which unreduced clonal embryo sacs may arise beside the reduced sexual megaspore. Such embryo sacs can be recognized cytologically, since a unique four-nucleate sac is produced (*2*), in contrast with the eight-nucleate sexual sac typical of the Gramineae. *Dichanthium*, a short-day plant, was selected because earlier experiments in controlled environments (*3*) had shown that the length of day at a critical phase during development of inflorescences controlled the incidence of apomictic embryo sacs.

I used two sources of material:

1) Strain CPI *14366*, a recent introduction from South Africa, is known to be a versatile apomict and is grown from seed at six stations differing in latitude along the eastern coast of Australia. The seed was obtained from plants grown in controlled environments under conditions promoting maximum apomixis (*3*).

2) Samples of approximately 12 inflorescences were collected at random at regular intervals from wild populations naturalized in northern Australia; all were at a similar stage of development, just preceding stigma exsertion. Inflorescences were immediately fixed in a mixture of ethanol and acetic acid (3 : 1 by volume) for 12 to 24 hours and stored in 70-percent ethanol. The cytological processing has been described (*3*), except that the material was embedded in Paraplast, and adhesion of sections to slides was facilitated by the amylopectin method of Steedman (*4*). The resultant data were based on analysis of at least 200 embryo sacs from four to six inflorescences for each sample. The embryo sacs were classified as sexual or apomictic according to earlier criteria (*3*). Approximately 5 to 10 percent of sacs, particularly those at a stage approaching maturation, could not be classified. Experiments in controlled environments have established that the minimum period for initiation and development of inflorescences is approximately 40 days (*5*), and this interval was used for extraction of the relevant climatic data.

Figure 1 shows the relative frequency of apomictic and sexual embryo sacs in inflorescences from a population of strain *14366* at stations between 9° and 36°S during first flowering in late summer—February 1964. Length of day during development of inflorescences strongly correlates with the incidence of apomixis. At the three southerly stations, where the day exceeded 14 hours throughout development of inflorescence, the incidence of apomictic sacs was low: 54.82 ± 3.46, 60.69 ± 3.72, and 63.08 ± 3.30 percent at stations *A*, *B*, and *C*, respectively. At the three northern stations, where photoperiods were less than 14 hours, it was high: 92.96 ± 1.41, 87.45 ± 2.01, and 91.40 ± 1.50 percent at stations *D*, *E*, and *F*, respectively. Other climatic factors, such as temperature, showed no clear association with the degree of apomixis.

Statistical analysis of these data indicates two quite distinct levels of apomixis, dependent on whether or not plants received photoperiods longer than 14 hours during floral development. Data for the three stations within each category were homogeneous [> 14 hours (*A,B,C*), $\chi^2 = 3.03$, with 2 degrees of freedom; < 14 hours (*D,E,F*), $\chi^2 = 5.54$, with 2 degrees of freedom], whereas the difference between the two categories was highly significant ($\chi^2 = 209.06$, with 1 degree of freedom). The mean percentage of apomictic embryo sacs for longer than 14 hours was 59.58 ± 2.03; for shorter than 14 hours, 90.84 ± 0.94. Geographically, this switch in the reproductive system occurred between latitudes 21° and 25°S, which is close to the latitude of origin of the race near Pretoria, South Africa (25°45′S).

The wild populations sampled were all tetraploid, some having a relatively constant high level (90 to 95 percent) of apomixis (see *6*). In other populations the degree of apomixis varied markedly during the flowering season. Figure 2 shows data from a ruderal population, *Mareeba-1*, from Mareeba, North Queensland (latitude, 17°00′S), where *D. aristatum* is known to have been naturalized for about 50 years. A sample collected in December, at the beginning of the flowering season in early summer, showed a low incidence of apomictic sacs (52.14 ± 4.22 percent), while a sample collected the following February (late summer) showed an increase to 78.09 ± 2.85 percent. Apomixis reached a maximum toward the end of the season, as judged by the April samples for 1963 and 1964: 90.71 ± 2.45 and 92.30 ± 1.98 percent, respectively.

The climatic data of Fig. 2 show that variation in photoperiod corresponds generally with patterns of temperature and rainfall, but unlike them it follows a precisely predictable course. Experiments in controlled environments (*3, 5*) have shown that apomixis in *D. aristatum* is controlled by the photoperiod during development of inflorescence; this evidence suggests that the seasonal variation in apomixis detected in *Mareeba-1* was controlled to a major extent by the lengths of day prevailing during floral development. The Decem-

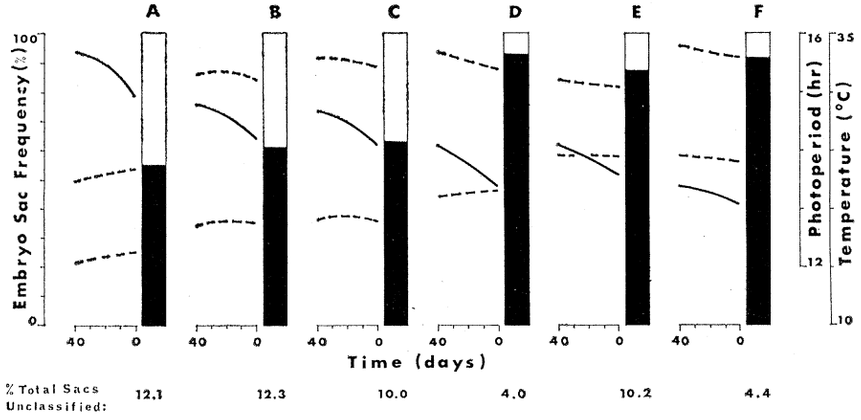

Fig. 1. Percentages of embryo sacs classified apomictic (black columns) or sexual (white columns), from samples collected at six stations during first flowering in February 1964, correlated with climatic factors during development of inflorescence (40 days before day of sampling). Photoperiods (including civil twilight), solid heavy lines; mean maximum and minimum monthly temperatures, broken lines. Stations, with latitudes: *A*, Bermagui, New South Wales, 36°28′S; *B*, Gatton, Queensland, 27°33′S; *C*, Gayndah, Queensland, 25°38′S; *D*, Mackay, Queensland, 21°12′S; *E*, Townsville, Queensland, 19°19′S; *F*, Port Moresby, New Guinea, 9°29′S.

ber sample, which showed a low level of apomixis, had been exposed to increasing photoperiods exceeding 13 hours during development of inflorescence. In contrast, the high values detected in the two April samples occurred when lengths of day were decreasing below 13 hours. The longer lengths of day followed conditions of stress during the dry season, and the lowest incidence of apomixis was recorded during this period. The samples showing high levels developed during conditions favorable for growth and flowering after summer rains and high temperatures.

Earlier observations by Nygren (*7*) indicated similar versatility in reproductive behavior in a Scandinavian race of the grass *Calamagrostis purpurea*, in which early panicles were sexual while the later ones were apomictic, but a cytologic analysis was not possible. Clausen's extensive studies of facultative apomixis in *Poa* (*8*) led to recognition of the potential of versatile apomixis as a breeding system. Apomictic reproduction allows rapid duplication of successful maternal genotypes, while, in combination with sexuality, the long-term benefit of genetic recombination is retained. The possibilities offered by environmental control have been reviewed (*9*). In 1959 Brooks (*10*) reported that environmental factors may influence apomixis in tetraploid races of *D. annulatum*, and experimental evidence is reported (*3*) of control of apomixis in *D. aristatum* by photoperiod.

Exposure of the same strain of *D.*

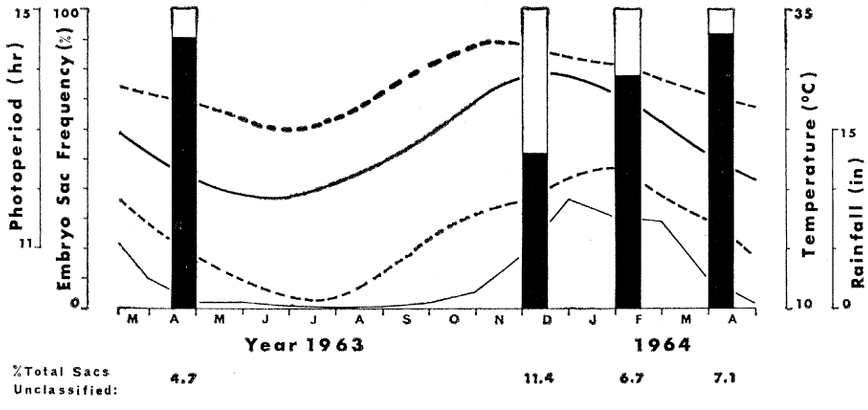

Fig. 2. Percentages of embryo sacs classified apomictic (black columns) and sexual (white columns) from samples collected during the flowering season from a wild population, *Mareeba-1*. Photoperiod (including civil twilight), heavy solid line; mean maximum and minimum monthly temperatures, broken lines; mean monthly rainfall, thin solid line.

aristatum to a range of climatic conditions over 27 degrees of latitude now demonstrates a marked association between length of day during development of inflorescence and the degree of apomixis. Furthermore, in a wild population of a genetically different strain, *Mareeba-1*, a change in the reproductive behavior of plants during the flowering season has been observed, the extent of apomixis again correlating with photoperiod. Such behavior is paralleled in the animal kingdom by many aphids (*11*), which undergo sexual reproduction during the short, cold days of winter, and apomixis by cyclic parthenogenesis during long, warm, summer days.

In addition, during development of inflorescence in *D. aristatum* there has evidently been a quantitative response to changes in length of day, as judged from Fig. 2. When the incidence of apomictic embryo sacs is compared further for a given range of photoperiod in the two strains investigated (Figs. 1 and 2), clear differences in response are evident. Such variation is akin to the differences in the critical length of day that control flowering in strains of the grass *Phleum pratense* (*12*).

R. B. KNOX

Department of Botany, Australian National University, Canberra

References and Notes

1. A. Gustafsson, *Lunds Univ. Arsskr. Avd. 2* **42**, 1 (1947).
2. H. E. Warmke, *Amer. J. Botany* **41**, 5 (1954); L. A. Snyder, A. R. Hernandez, H. E. Warmke, *Botan. Gaz.* **116**, 209 (1955); W. V. Brown and H. P. Emery, *Amer. J. Botany* **45**, 249 (1958).
3. R. B. Knox and J. Heslop-Harrison, *Botan. Notiser* **116**, 127 (1963).
4. H. F. Steedman, *Nature* **179**, 1345 (1957).
5. R. B. Knox, in preparation.
6. J. R. Harlan, *Amer. Naturalist* **97**, 91 (1963).
7. A. Nygren, *Hereditas* **37**, 519 (1951).
8. J. Clausen, *Proc. Intern. Congr. Genetics 9th Caryologia Suppl.* **6**, 469 (1954); ———, and W. M. Hiesey, *Rept. Scottish Plant Breeding Sta.* **1958**, 41 (1958).
9. J. Heslop-Harrison, in *Recent Advances in Botany* (Toronto Univ. Press, Toronto, 1961), vol. 1, pp. 891–95; in *Advances in Ecological Research* (Academic Press, New York, 1964), vol. 3, p. 159.
10. M. H. Brooks, *Proc. Intern. Congr. Genetics 10th* **2**, 36 (1958).
11. S. Marcovitch, *J. Agr. Res.* **27**, 513 (1924); A. D. Lees, *J. Insect Physiol.* **3**, 92 (1959); D. H. R. Lambers, *Ann. Rev. Entomol.* **11**, 47 (1966).
12. M. W. Evans and H. A. Allard, *J. Agr. Res.* **48**, 571 (1934).
13. I thank J. Heslop-Harrison for suggesting this project; L. T. Evans and L. D. Pryor for encouragement and for criticizing the manuscript; R. W. Downes, J. C. Tothill, D. Cameron, G. P. Keleny, and D. I. Sillar for collecting samples; O. R. Byrne for statistical analysis; and E. Chapman for technical assistance. Work began during tenure of a NATO research fellowship of the U.K. Science Research Council at C.S.I.R.O. Division of Plant Industry, Canberra, Australia.

13 April 1967