Department of Environmental Biology, Department of Botany, The Arboretum, University of Guelph, Guelph, Ont., Canada

Pollen dimorphism and dioecy in Vitis aestivalis

by

P. G. KEVAN, D. C. A. BLADES, U. POSLUSZNY and J. D. AMBROSE

Dimorphisme du pollen et dioécie chez Vitis aestivalis

Sommaire: La vigne d'été, *Vitis aestivalis* est une plante rare au Canada. Nous avons découvert que la population sud-ontarienne de cette espèce est dimorphique, c'est-à-dire mâle et femelle. Les fleurs des plantes mâles produisent du pollen «tricolporate». Ce pollen est plus gros que le pollen «inaperturate» des plantes femelles. Les fleurs entières des deux formes sont différentes; chez les mâles, les étamines sont bien développées et le pistil est sous-développé, tandis que chez les femelles la situation contraire se produit. Nos conclusions nous portent à croire que cette espèce est dioïque comme toutes les autres vignes sauvages. Ces résultats sont significatifs en ce qui a trait à la viticulture et aux programmes d'amélioration des vignes.

Key words: pollen, morphology, flower, sexuality, Vitis, Canada.

Introduction

Dioecy in grapes is poorly documented and understood. Most commercial varieties of *Vitis vinifera* L. have hermaphroditic flowers and are thought to be self-pollinating. *Vitis vinifera* ssp. *silvestris* is functionally dioecious (Brantjes 1978) as are other wild grapes such as *V. riparia*, *V. cordifolia* and *V. rotundifolia* (see Kevan *et al.* 1985). Because commercially grown grapes have their ancestry in various selections and hybridizations of wild grapes, Kevan *et al.* (1985) suggested that some of the problems of low production and sterile pollen (e.g. Lombardo *et al.* 1976, 1978; Ahmedullah 1983; Me *et al.* 1984) may be related to the expression of dioecy, which is known to occur occasionally (Meehan 1867 a, b). The genetic mechanisms in the expression of sexual polymorphism in grapes are complex and not well understood (see Antcliff 1980). We believe that a better understanding of sexual expression in wild grapes will assist in understanding the outcomes of breeding programs, hybridizations, and the reasons for some instances of low productivity.

Vitis aestivalis, the summer grape, is a rare plant in Canada (Argus and White 1977), occurring only in a few locations in southern Ontario. Unlike *V. riparia* which grows on forest margins, fence rows, and even sandy lake shores, *V. aestivalis* typically grows within forests. The aim of this study was to investigate the sexual expression in *V. aestivalis* in Ontario and to compare our findings with those of Kevan *et al.* (1985) on *V. riparia*.

Materials and methods

Our study site was at Fireman's Park, Niagara-on-the-Lake, Ontario (43° 8' N, 79° 7' W). The plants were large, each with numerous, stout vines up to 8 cm in diameter, and mostly extending into the forest canopy some 15—20 m overhead. In a few places, where trees had fallen, the vines' foliage and flowers were accessible from the ground.

In June and July 1987, we visited Fireman's Park to inspect the vines for flowers. A population survey and a collection of flowers was done during peak flowering on 19 June, 1987. Flowers of each sex were preserved in FAA (10 % formalin: glacial acetic acid: 95 % ethanol 1:1:9) and taken to the laboratory for examination. Some flowers were prepared by critical point drying and sputter coating with gold-palladium for scanning electron microscopy by Hitachi S-570. Pollen from each kind of flowers was placed on microscope slides and wet mounted under a cover slip for examination by light microscopy.

Results

Two kinds of flowers were found on separate individuals of *V. aestivalis*. One flower type had well developed stamens with a small ovary (Fig. 1), and the other poorly developed stamens and a large ovary with prominent stigma (Fig. 2). This dimorphism is identical to that found in *V. riparia* so the forms were designated male and female respectively. The tangles of vines and the inaccessibility of the flowers in the canopy made it impossible to determine sex ratio of the population. However, we were confident that we found six different male plants and one female plant.

Further evidence of sexual dimorphism in V. aestivalis came from examination of pollen from the two flower types collected. We found the same clear differences as were found in V. riparia (Kevan et al. 1985); the pollen of male flowers was spherical and tricolporate (mean diameter = 26.3 μ m, 95 % confidence interval \pm 0.5, N = 100; Fig. 3), whereas pollen from female flowers was spherical, but completely inaperturate (mean diameter = 23.7 μ m \pm 0.4, N = 100; Fig. 4). The sizes of the pollen grains from the two sexes are highly significantly different (t = 7.65, P < 0.001). Revisiting Fireman's Park on August 19, 1987, we were unable to find any fruit on the plants we had seen before. Because the males would not be expected to set fruit, and the single female had had only a few inflorescences within sight, we are unable to offer the corroborating evidence of dioecy.

Discussion

Although we have not undertaken definitive experiments, like those used for *V. riparia* (Kevan *et al.* 1985) to prove dioecy in *V. aestivalis*, we find the dimorphism of structural characteristics of the flowers and of the pollen grains similar to those of *V. riparia* and indicate the same breeding system. The sex ratio of the dioecious plants

Fig. 1—4: Photographies prises à l'aide d'un microscope électronique à balayage. L'échelle des barres dans les Fig. 1 et 2 est de 1 mm. Celle des barres dans les Fig. 3 et 4 est de 6 μm.

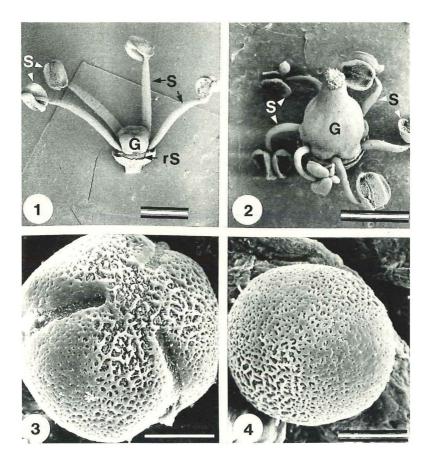
Fig. 1: Vue supérieure oblique d'une fleur mâle démontrant un gynécée avorté (G) et des étamines (S) aux filaments droits. Une étamine a été enlevée (rS) pour mieux montrer le gynécée.

Fig. 2: Vue supérieure oblique d'une fleur femelle démontrant un gynécée complètement développé (G) et des étamines (S) aux filaments récurvés.

Fig. 3: Vue supérieure d'un grain de pollen «tricolporate» provenant de l'étamine d'une fleur mâle.

Fig. 4: Un grain de pollen «inaperturate» provenant de l'étamine d'une fleur femelle.

is usually skewed in favour of males (OPLER and BAWA 1978) as is the case of *V. riparia* (KEVAN *et al.* 1985) and is suggested by our minimal data on *V. aestivalis*. The difference in the sizes of the pollen grains in *V. aestivalis* is unlike the situation in *V. riparia* with pollen grains of each sex being the same size (KEVAN *et al.* 1985). Perhaps the small, but noteworthy difference in pollen dimorphism between *V. riparia* and *V. aestivalis* signifies a greater development of dioecy in the latter. Certainly, we conclude that *V. aestivalis*, like *V. riparia*, is truly dioecious.



Figs. 1—4: Scanning electron micrographs. The scale bars in Figs. 1 and 2 equal 1 mm, in Figs. 3 and 4 the bars equal 6 μ m.

Fig. 1: An oblique top view of a male flower showing the aborted gynoecium (G) and Stamens (S) with upright filaments. One stamen has been removed (rS) in order to show the gynoecium.

Fig. 2: An oblique top view of a female flower showing a fully developed gynoecium (G) and stamens (S) with recurved filaments.

Fig. 3: A top view of a tricolporate pollen grain from the stamen of a male flower.

Fig. 4: An inaperturate pollen grain from the stamen of a female flower.

Summary

- Problems of low production and sterile pollen in varieties of *Vitis vinifera* may be tracable to their ancestral relations with dioecious wild grapes.
- 2. Like *V. riparia*, the wild summer grape *V. aestivalis* has dimorphic male and female flowers; but unlike *V. riparia* it also has dimorphic pollen grains with the pollen from the female flower being significantly smaller.
- 3. It seems fairly certain that *V. aestivalis* is truly dioecious.

Acknowledgements

This work was supported in part by a Strategic Grant from the Natural Sciences and Engineering Research Council of Canada to Usher Posluszny and Peter G. Kevan and from the World Wildlife Fund to John D. Ambrose and Peter G. Kevan. We would like to thank the excellent technical assistance of Pamela F. Jacobs and Lesley S. Smith in the preparation of the S.E.M. micrographs. As well, we are grateful to Terry G. Grignon who helped with the statistical analysis and the preparation of the manuscript and Christian R. Lacroix who did the french translation.

Literature cited

Ahmedullah, M.; 1983: Pollen morphology of selected *Vitis* cultivars. J. Amer. Soc. Hort. Sci. 108, 155—160.

ANTCLIFF, A. J.; 1980: Inheritance of sex in Vitis. Ann. Amélior. Plantes 30, 113—122.

Argus, G. W.; White, D. J.; 1977: The rare vascular plants of Ontario. Syllogeus (Can. Natl. Mus. Nat. Sci.) 14.

Brantjes, N. B. M.; 1978: Pollinator attraction to Vitis vinifera ssp. silvestris. Vitis 17, 229-233.

Kevan, P. G.; Longair, R. W.; Gadawsky, R. M.; 1985: Dioecy and pollen dimorphism in *Vitis riparia* (Vitaceae). Can. J. Bot. 63, 2263—2267.

- Lombardo, G.; Cargnello, G.; Bassi, M.; Carraro, L.; 1978: Pollen ultrastructure in different vine cultivars with low productivity. Vitis 17, 221—228.
- — ; Carraro, L.; Carrallo, G.; Bassi, M.; 1976: Ultrastructure of pollen of *Vitis vinifera* L. cv. 'Picolit giallo' and its behaviour in experiments of self- and cross-pollination. Vitis 15, 73—81.
- Me, G.; Sacerdote, S.; Vallania, R.; 1984: Osservazioni sulla meiosi in cellule madri del polline di Vitis vinifera L. (cv. Barbera) diploide e tetraploide. Vitis 23, 195—201.
- MEEHAN, T.; 1867 a: Note on dioecious forms of *Vitis vinifera* L. Proc. Acad. Nat. Sci. Philadelphia **1867** (April), 42.
- ; 1867 b: Additional note on dioecious forms of Vitis vinifera L. Proc. Acad. Nat. Sci. Philadelphia 1867 (April), 98—99.
- OPLER, P. A.; BAWA, K. S.; 1978: Sex ratios in tropical forest trees. Evolution (Lawrence, Kansas) 32, 812—821.

Eingegangen am 28. 4. 1988

Dr. P. G. KEVAN
Department of Environmental Biology
University of Guelph
Guelph, Ontario N1G 2W1
Canada

Note added in proof:

In 1988, we found a small population of *V. aestivalis* in La Salle, Ontario. The plants were clearly male or female with flowers corresponding to those described from plants at Fireman's Park