Enod2 exhibits linkage with *a* (linkage group IA) and *Fum* (linkage group II)

Paruvangada, V. G., Weeden, N.F.,   Department of Horticultural Sciences
Cargnoni, T. and Yu, J.   NYSAES, Cornell University
                                                               Geneva, NY 14456, USA

Gorel', F.   Institute of Cytology and Genetics
                                                            Novosibirsk 630090, Russia

Frew, T., Timmerman-Vaughan, G.M. and   Crop and Foods Research
McCallum, J.   Private Bag 4704, Christchurch, New Zealand

The gene *a* traditionally has been placed on the upper portion of linkage group I (2, 11). However, recent work by Kosterin and others (4, 5) has indicated linkage group I may actually represent two linkage groups, tentatively designated IA and IB. Linkage group IB covers 70 to 150 cM, stretching from the *d — sym2* region to *af— i* and perhaps somewhat beyond (3, 11). Linkage group IA includes *Aat-p, His2-6, a, lf,* and *His7,* but is relatively short (50-100 cM) and thus a good candidate for synteny with one of the other linkage groups described for pea. Here we present results identifying linkage between a nodulin gene, *Enod2,* and markers on both linkage group IA and II, suggesting that these two linkage groups may be on the same chromosome.

The Enod2 clone was generously supplied by T. Bisseling (Agricultural University, Wageningen). Another DNA clone, CD40 (=*Lg-J*), was mapped by Ellis et al. (3). The *Enod2* gene codes a hydroxyproline-rich protein expressed early in nodule development (10); however, the specific function of the protein has yet to be determined. The probe was radiolabelled and used for Southern hybridization analysis as described in Polans et al. (7). Two populations were examined. A set of F_8 recombinant inbred lines (RILs) from the cross JI1794 x Slow and a second set of 60 F_6 RILs from the cross Sparkle x WL1238. In both populations polymorphism was observed using the restriction endonuclease, *Eco*RI.

In both populations, *Enod2* gave approximately a 1:1 segregation ratio (18:25 in JI1794 x Slow and 22:20 in Sparkle x WL1238), as did most of the several hundred other markers scored (data not presented), indicating that there were no major differences in karyotype between each pair of parents. In the JI1794 x Slow population, joint segregation analysis for *Enod2* and *Fum* gave 11 recombinants out of the 42 lines scored for both markers and the analysis for *Enod2* and *His2-6* gave 14 recombinants out of 42 lines. Using the equation r = R/[2-2R] (8), which relates recombination frequency (r) to the proportion of RILs in which recombination has occurred between two loci (R), we obtained a recombination frequency between *Enod2* and *Fum* of 18% and one between *Enod2* and *His2-6* of 25%. Although these linkages are not particularly tight, we have located several other RFLP and RAPD markers in the region, generating a much more saturated map and confirming the linkages (Fig. 1).

In the Sparkle x WL1238 RIL population *Enod2* again showed linkage with markers on linkage group IA : *a* - 20% - B319 - 9% - *Enod2*. However, in this population we did not observe linkage between *Enod2* and the markers on linkage group II including *wb, k,* and *Pgm-p.* These latter markers are over 20 cM from *Fum* on the side opposite *Enod2,* and the relatively few RILs in the population may have prevented our observing significant deviations
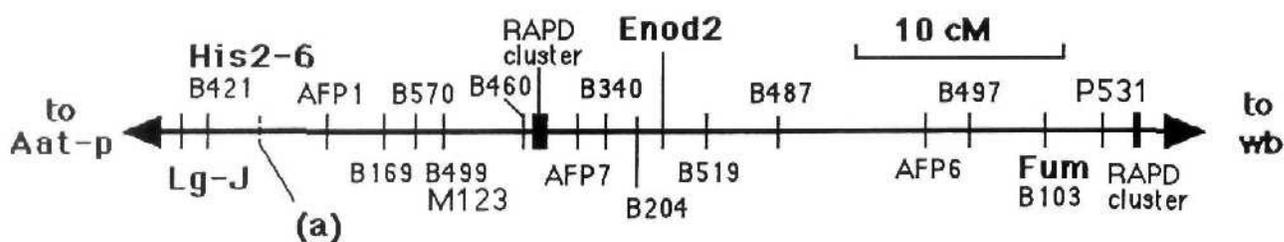
Fig. 1. Linkage group for the JI1794 x Slow population that contains *Enod2* and markers on both linkage group IA *(His2-6, Lg-J)* and on linkage group II *(Fum)*. These standard markers are given in bold print, two cDNA markers (M123 and P531) are given in medium sized print, and RAPDs are given in small print. Two RAPD clusters containing 5 or more RAPDs each, are also mapped. The approximate position of gene *a* is shown for convenience, although it did not segregate in this population.

from random assortment Unfortunately, *Fum* was not segregating in this population, nor have we identified many DNA markers in this region of the genome in this set of RILs. *Enod2* did not display significant linkage with any other region of the genome in either of the RIL populations.

       We conclude that *Enod2* is located 25 to 30 cM from *a* on linkage group IA. Our results support the suggestion by Kosterin that *a* and *d* are located on different linkage groups, because *Enod2* did not display linkage with any marker on linkage group IB. Rather, it appears that group IA is syntenic with *Fum,* forcing the joining of group IA with linkage group II. On cytogenetic grounds such a merger would be acceptable because group IA is assigned to chromosome 6, but appears to constitute only a portion of this chromosome, whereas group II has not been assigned to a chromosome (9).

       The location of *Enod2* does not correspond to that of any previously mapped *sym* or *Enod* gene (6). Two *sym* genes *(sym14* and that from JI1794, tentatively designated *Sym22)* are near *Fum,* but appear to be on the side of *Fum* opposite to *Enod2* (S. Temnykh, T.A. LaRue, and N.F. Weeden, unpublished). Another non-nodulating gene, *Twt,* lies very near *a* (1). Thus, *Enod2* must be a new symbiosis gene that can not be paired with any of the known symbiosis mutants.

1.   Berdnikov, V.A., Gorel', F.L. and Temnykh, S.V. 1994. Pisum Genet. 26:9-10.
2.   Blixt,S. 1972. Agri Hort. Genet. 30:1-293.
3.   Ellis, T.H.N., Hellens, R.P., Turner, L., Lee, C, Domoney, C. and Welham, T. 1993. Pisum Genet. 25:5-12.
4.   Kosterin, O.E. 1993. Pisum Genet. 25:23-26.
5.   Kosterin, O.E. and Rozov, S.M. 1993. Pisum Genet. 25:27-31.
6.   LaRue, T.A. and Weeden, N.F. 1992. Pisum Genet. 24:5-12.
7.   Polans, N.O., Weeden, N.F. and Thompson, W.F. 1985. Proc. Natl. Acad. Sci. USA 82:5083-5087.
8.   Taylor, B.A. 1978. *In:* Origins of Inbred Mice. H.C. Morse (ed.). Academic Press, New York, pp. 432-438.
9.   Temnykh, S.V. and Weeden, N.F. 1993. Pisum Genet. 25:1-3.
10. van der Weil, C., Sheres, B., Franzen, H., van Lierop, M.-J., van Lammeren, A., van Kammen, A. and Bisseling, T. 1990. EMBOJ. 9:1-7.
11. Weeden, N.F., Ambrose, M. and Swiecicki, W.K. 1991. Pisum Genet. 23:cover.