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Comment

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In a recent article in *Biology Letters*, Dudley & File (2007) claim to have demonstrated kin recognition and altruistic behaviour towards related individuals in an annual plant. This is an extraordinary claim and, if true, has far-reaching implications for many topics in plant ecology and evolutionary biology. Unfortunately, this claim cannot be substantiated with the data that they have presented.

Dudley & File's argument rests entirely on the detection of a shift in allocation between plants grown with kin versus non-kin individuals. However, there are serious conceptual challenges involved in documenting these types of allocational shifts (McCoy *et al.* 2006).

Before an adaptive allocation response can credibly be invoked, an important null hypothesis to exclude is that of an ontogenetic change in biomass allocation with organism size (McConnaughay & Coleman 1999). One way to correct for these effects is to use an analysis of co-variance (ANCOVA) to compare main effects across a range of sizes. However, ANCOVA is a very limited type of analysis. It is bound by all of the assumptions of analysis of variance (ANOVA) and one more: that the slope of the relationship between the response and the covariate does not differ among treatment groups (Sokal & Rohlf 1995). Just as in ANOVA, the test of a main effect is a test for an additive difference among treatment groups. A significant interaction of the main effect and the covariate indicates that the relationship of the response variable to the covariate differs among the treatment groups, and thus no additive difference can be attributed to the main effect.

The significant interaction of 'kin' and 'logleaf' in table 1 of Dudley & File (2007) indicates that the slopes of the relationship between size and fine root allocation do differ between the kin and non-kin groups. The significance of the main effect is therefore not interpretable as a simple additive difference among treatments, and the least-squares means reported are artificial as they are based on the false assumption of a common slope among groups. Unfortunately, the underlying allometries for the kin and non-kin treatments are neither reported nor shown graphically, and it is therefore impossible for the reader to determine whether the ANCOVA result actually supports the conclusion presented.

The analyses presented in this study also employ a response variable that fails to capture the observations of interest (the allocation patterns of individual plants). Dudley & File (2007) calculated biomass ratios at the pot level, by dividing summed above-ground biomass of the four plants in each pot by the summed below-ground biomass of those plants (r.h.s. of equation (1.1)). This does not give the same result as taking the average of the ratios observed for individuals within a pot (l.h.s. of equation (1.1)), which would be the appropriate value for this type of analysis,

$$\frac{\left(\frac{a_1}{b_1}\right)+\left(\frac{a_2}{b_2}\right)+\cdots+\left(\frac{a_j}{b_j}\right)}{j} \neq \frac{a_1+a_2+\cdots+a_j}{b_1+b_2+\cdots+b_j}. \qquad (1.1)$$

The method employed is not only inaccurate but also biases the response variable towards the ratios obtained by the largest individuals within each pot. The potential for this problem to result in spurious results is particularly acute given that the final sizes of plants grown with root neighbours varied over an order of magnitude (figure 2 in Dudley & File (2007)).

Even if an allometric shift could be demonstrated with these data, the causal link to altruistic behaviour in plants is tenuous. The purported allometric shift is suggested to function in reducing competition and thereby maximizing inclusive fitness among siblings, but competition is not apparent in the data presented. Figure 1*b* in Dudley & File (2007) shows that plants grown with neighbours grow substantially larger than plants grown alone, and, contrary to expectation, the largest plants appear to have been non-kin neighbours, although the experiment apparently lacked sufficient power to detect a difference in final plant size. Neither is there an obvious effect of competition in the reproductive allocation data shown in figure 2 in Dudley & File (2007).

The comparison of root ratios and sizes between neighbour and solitary plants is also problematic because it has been demonstrated repeatedly that differences in pot size, even when per plant soil volume is held constant, can affect plant growth and allocation patterns (NeSmith & Duval 1998). If uncontrolled, this effect can confound the interpretation of neighbour effects such as the ones shown in this experiment (Hess & de Kroon 2007; Semchenko *et al.* 2007).

Finally, the large variation in final plant size of neighbour plants versus those grown alone apparent in figure 2 in Dudley & File (2007) suggests the possibility that if plants did compete, competition was strongly asymmetric within pots. If general across kin treatments, this would undermine claims of sibships acting in concert to maximize inclusive fitness at the expense of individual growth. If not, combined with the bias introduced by the improper calculation of the root ratios, this could lead to the result observed in Dudley & File's ANCOVA analysis.

At the very least, a more detailed exposition of the data presented in Dudley & File's paper is needed before their argument for kin recognition in plants can be accepted.

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The accompanying reply can be viewed on page 69 or at http://dx.doi.org/doi:10.1098/2007.0585.

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