of other researchers when the correct comparisons are made, that is, those which take into account differences between the volatile anesthetic agents and the means by which they are administered. The conflict arises when a random comparison of results is made.

Another point brought up by Strum *et al.* concerns the spatial origin of the fluorine-19 signal observed in our NMR experiments. Strum *et al.* suggest that "the focus of the NMR surface coil may have provided images of isoflurane in fat rather than brain." It is important to note that the data we have reported are based upon fluorine-19 spectra obtained with a surface coil, and are not from NMR images. These and other studies (*7*) in our laboratory using fluorine-19 rotating-frame zeugmatography, imaging, and spatially localized relaxation time methods indicate that the region sampled is indeed within the brain. These results were corroborated by in vitro experiments on isoflurane and halothane distribution in brain and other tissues. The data of Strum *et al.* show longer retention times in fat than in brain or muscle, which agrees with our own results. Thus, they report for isoflurane in fat a decrease of 60% at 270 minutes, whereas we observe in that time an 85% decrease in brain concentration (*8*). The loss of isoflurane we reported is greater than the loss reported for fat by Strum *et al.* Therefore, it would be difficult to conclude that our detected NMR signal originated from fatty tissues.

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8. In their figures 1 and 2, Strum *et al.* cite one anomalous value for isoflurane elimination taken from an abstract submitted prior to an Anesthesiology Society meeting where more complete data were presented.

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Mediation of Interactions Among Insect Herbivores

Raupp *et al.* (*1*) describe experiments that purport to demonstrate that methylcyclopentanoid monoterpenes released from exocrine glands mediate interactions between larvae of the leaf beetle, *Plagiodera versicolora*, and larvae of another insect herbivore. While the conclusion that the secretion mediates these behaviors is valid, the assumption that the methylcyclopentanoid terpenes are responsible is not directly demonstrated.

The authors do not appear to sufficiently acknowledge that the larval exocrine secretions of *Plagiodera* are complex biphasic mixtures. Sugawara (*2*), in one of the few quantitative studies in this area, showed that less than 10% of the larval secretion of *Plagiodera versicolora distincta* is nonaqueous (4.6 mg extracted in pentane from 50.2 mg of crude larval secretion). Similar data were given for two other species (*2*). By using only the natural secretion in their bioassays, Raupp *et al.* do not appear to justify their title "Methylcyclopentanoid monoterpenes mediate interactions among insect herbivores." They may have verified that methylcyclopentanoid monoterpenes were present in the secretion, but they did not employ standard compounds in their behavioral tests to prove that the monoterpenes themselves elicited the behaviors observed. Authentic compounds do not appear to have been used as controls in the bioassays or as standards for analysis of the extract. In fact, the mass spectroscopic data differed from that previously reported for *P. versicolora* (*3*).

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Response: Duffield and Wheeler (*1*) make an interesting comment concerning the methodology used to assay the biological activity of leaf beetle defensive secretions. The defensive secretions of many Chrysomelinae larvae, including the one we studied, are biphasic blends of compounds. Attention to the biological activities of these secretions has focused primarily on the naturally occurring blends or the nonpolar components found in the mixture (*2–5*). Little work has been done on the biological activity of the aqueous fraction of these secretions. However, we have good reason to believe that the nonaqueous components such as the methylcyclopentanoid monoterpenes are biologically active for the following reasons. At least three recent studies have examined the biological activity of natural secretions or derivatives of secretions of Chrysomelinae beetles. The larvae of *Chrysomela vigintipunctata costella, C. populi*, and *Gastrolina depressa* produce salicylaldehyde and benzaldehyde, salicylaldehyde alone, and juglone alone, respectively, in the nonaqueous portion of their defensive secretions. Bioassays of larval secretions of the predatory ant *Lasius niger* indicated that the biological activity of the natural parent secretion was identical to that of each isolated nonpolar component (*4*). In another study salicylaldehyde was found to be biologically active against the predatory ant *Myrmica rubra* (*5*). Salicylaldehyde is a nonaqueous component in the defensive secretion of at least eight species of Chrysomelinae found worldwide (*6*). Also, Duffield and his colleagues (*3*) demonstrated in a recent study that the natural larval secretion of the Chrysomelinae leaf beetle *Gastrophysa cyanea* was strongly repellent to a predator, the fire ant *Solenopsis invicta*. They went on to demonstrate that one of the cyclopentenic monoterpenes found in the secretion elicited the same avoidance response by fire ants as did the natural blend. We concede that the title of our report may have been too specific because we did not test individual components in the secretion. However, the weight of the evidence clearly indicates that the isolated nonaqueous compounds found in these secretions have the same biological activity as the natural parent secretion. Duffield and Wheeler are correct in pointing out that we know very little about the composition or biological activities of the aqueous fractions of these secretions. This criticism holds for all the studies described above.

Finally, Duffield and Wheeler note that our mass spectroscopic data did not indicate the presence of chrysomelidial as did one other previous account for a North American population (*7*). However, they do not mention that other groups of scientists studying secretions of *P. versicolora* did not find chrysomelidial for populations in North America, Europe, and Japan (*6, 8*). Recent-

ly, it has been suggested that the presence of chrysomelidial in the secretion of *P. versicolora* may be artifactual, resulting from a decomposition of plagiodial (*6*). Our analysis indicated the presence of plagiolactone in the secretion. Contrary to Duffield and Wheeler's statement, this agrees well with other published accounts, including the one by Meinwald *et al.* (*6–8*).

Until such time as aqueous fractions of the defensive secretions of these larvae are assayed, we are forced to assume, as have many others (*2–6, 8*), that there is a strong causal relationship between the biological activity of the larval defensive secretions and the nonaqueous compounds they contain.

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Histones and Metal-Binding Domains

Jeremy M. Berg (*1*) describes potential metal-binding domains in nucleic acid–binding proteins. These metal-binding domains consist of sequences Cys-X_{2-4}-Cys-X_{4-15}-His-X_{2-8}-Cys. Berg also cites the fact that proteins known to bind ions have short sequences of the form Cys-X_{2-4}-Cys, Cys-X_{2-4}-His, or His-X_{2-3}-His. A well-known DNA-binding protein, histone H3, also has the conserved sequence Cys-Ala-Ile-His from residues 110 to 113 (*2*). The nucleosome octamer has two histones of each kind of four histones. These histones are organized about an axis of symmetry, such that these conserved sequences on each histone H3 oppose each other (*3*). The apposition of these two histone H3 sequences would generate a structure potentially able to coordinate a metal ion, namely 2(Cys-Ala-Ile-His).

It has been proposed that the two histones H3, in the nucleosome, interact through the formation of an interchain disulfide bond (*3*). The suggestion that the two copies of histone H3 may interact through the coordination of metal ions raises the possibility that other types of interactions may occur within nucleosomes. The availability of different forms of interaction may also have implications for nucleosome structure and flexibility.

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Response: Saavedra points out the occurence of the sequence Cys-X_2-His in histone H3 and suggests that these residues are potentially involved in binding a metal ion between two H3 subunits. Such a sequence would not have been detected by the search algorithm I described (*1*) and, indeed, a general search for such short sequences would undoubtedly pick up many sequences not involved in metal binding. However, as Saavedra notes, these residues do indeed lie at the H3 dimer interface as demonstrated by observations with disulfide-linked H3 dimers (*2*) and by the 3.3 Å resolution electron density map of the histone octamer (*3*). The involvement of residues from individual polypeptides in binding metal ions and stabilizing an oligomer is well precedented by the Zn^{2+}-based insulin hexamer (*4*). However, it must be kept in mind that with histone H3, as with the proteins identified by the previous search, the sequences noted are potential metal-binding sites. Demonstration of the actual role(s) of metal binding in these systems will require careful experimental investigation.

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