Hormonal modification of resource sharing in the clonal plant *Fragaria chiloensis*

P. ALPERT†, C. HOLZAPFEL and J. M. BENSON

*Biology Department, University of Massachusetts, Amherst, MA 01003-5810, USA*

**Summary**

1. Clonal plants can share resources such as carbon and nitrogen among connected, separately rooted plant units or ramets. In many cases, resource sharing tends to equalize the performance of ramets, suggesting that differences in resource supply drive the sharing process, with resources moving from ramets with high access to resources to those with low access to resources. However, some patterns of apparent C and N sharing in the stoloniferous wild strawberry *Fragaria chiloensis* make the growth of ramets less equal, which is inconsistent with this model of resource sharing. As hormones are a mechanism by which non-clonal plants can make the growth of the branches of a shoot less equal, we hypothesized that clonal plants might use hormones to modify patterns of resource sharing between ramets.

2. Auxin (IAA) or cytokinin (BA) was applied to the shoot tip of ramets using a non-invasive protocol previously shown to increase internal hormone concentrations. $^{14}$C or $^{15}$N was then fed to connected ramets, and the amounts of $^{14}$C or $^{15}$N imported by the hormone-treated ramets and by suitable controls were measured.

3. Ramets treated with IAA showed greater C import from connected ramets than did control ramets when the IAA-treated and control ramets were shaded and the connected ramets were not. Ramets treated with IAA showed increased N import from connected ramets compared with the controls when the IAA-treated and control ramets were given less N than the connected ramets, and were also younger than the connected ramets. IAA had no consistent effect on C or N sharing between ramets given the same resource treatments as one another and did not affect the direction of N sharing, which is strongly acropetal in *F. chiloensis*. BA showed little effect on resource sharing.

4. The results supported the hypothesis that hormones can modify patterns of resource sharing between ramets in clonal plants. In *F. chiloensis*, auxin can enhance resource import by ramets in relatively resource-poor microsites. Clonal plants may use hormones as a mechanism for physiological integration of connected ramets.

**Key-words:** Auxin, carbon translocation, cytokinin, nitrogen transport, physiological integration between ramets


**Introduction**

Resource sharing, the ability to transfer water, carbon compounds and mineral nutrients between connected, asexual offspring (ramets) of the same clone, is an important advantage of the clonal growth form in plants (Alpert 1996; de Hutchings 1999; Jónsdóttir & Watson 1997; Kroon et al. 1996; Stuefer & Hutchings 1994). When connected ramets experience different levels of resource availability or herbivory, resource sharing can significantly increase their individual and combined survival, growth and reproduction (Alpert & Mooney 1986; Hutchings & Wijesinghe 1997; Lau & Young 1988; Stuefer et al. 1996; Wijesinghe & Handel 1994).

Typically, clones show net transfer of resources from ramets in relatively resource-rich microsites to those in relatively resource-poor microsites (Hutchings 1999; Hutchings & Wijesinghe 1997; Stuefer & Hutchings 1994). Carbon sharing is often facultatively bidirectional, with little sharing in the absence of resource patchiness, and sharing either from older to younger ramets (acropetal transport through connections) or from younger to older ones (basipetal transport), depending on relative shading or defoliation (Alpert & Mooney 1986; Hutchings 1999; Jónsdóttir & Watson 1997). Nitrogen sharing tends to be more constrained.
some species, including the stoloniferous herb *Fragaria chiloensis* (L.) Duchesne, N sharing is almost entirely from older to younger ramets, regardless of resource gradients (Alpert 1996; Marshall & Anderson-Taylor 1992).

Whereas the effects and patterns of resource sharing in a number of clonal plant species are well documented, the physiological mechanism for resource sharing has been little explored. The simplest assumption is that differences in resource availability to different ramets create internal gradients in resource concentrations that drive net resource transfers between ramets (e.g. Marshall 1990), subject to vascular constraints (Lötscher & Hay 1996; Marshall & Price 1997). Sink–source models (e.g. Kaitaniemi & Honkanen 1996) based on this assumption are consistent with the tendency of resources to move into relatively resource-poor ramets and with the most common effect of connection on ramets, which is to make their total biomass more equal (Hutchings & Wijesinghe 1997; Jónsdóttir & Watson 1997; Marshall 1990).

However, gradient-driven resource transport does not account for at least two patterns of resource sharing observed in *F. chiloensis*. First, connection between ramets in microsites with different levels of soil nutrients can cause their biomass to be less equal (‘rich get richer’ effect; Alpert 1996). Second, connection between a shaded ramet given high N and a ramet given high light and low N can reverse rather than reduce differences between ramets in biomass (‘over-sharing’; Alpert 1999). Some patterns of apparent resource sharing in other clonal species also run counter to the expectation that resources always move from ramets with high to those with low resource supply (e.g. Matlack 1997).

Hormonal modification of resource transport between ramets is an alternative mechanism that could account for these patterns. There is considerable evidence in non-clonal species that hormones can cause differences between biomass of plant parts to increase in response to differences in resource availability. Hormones regulate translocation between branches within shoots (e.g. Voesenek & Blom 1996) and between symplast and xylem (De Boer 1999); they are transported in phloem (Baker 2000; Hoad 1995); and they mediate many environmental effects on plant development (e.g. Tamas 1995). Auxin can directly transport carbohydrates towards apical meristems (e.g. Morris & Arthur 1987), and cytokinin can cause the preferential translocation of nutrients and organic compounds to cytokinin-treated tissues (Arteca 1996). Auxin and cytokinin can control differentiation and regeneration of tracheary cells and sieve-tube elements (Jacobs 1998; Sachs 1991), resulting in the diversion of water and nutrients from shaded to unshaded branches within a shoot (Sachs & Novoplansky 1997). As hormones such as auxins are mainly produced by growing shoots, it may be unlikely that they can trigger changes in resource sharing independent of growth, but it seems very possible that they could play a role in the regulation of resource sharing.

We therefore hypothesized that clonal plants can use hormones to modify patterns of resource sharing between connected ramets, and predicted that increasing hormone concentration in one ramet of *F. chiloensis* would increase its import of resources from a connected ramet. Our three main subsidiary questions were: (i) Can hormones increase resource import when ramets experience similar resource availabilities? (ii) Can hormones increase resource import by ramets that experience relatively low resource availabilities? and (iii) Are hormones responsible for directional constraints on nutrient sharing? We tested the effects of two major types of plant hormone, auxin and cytokinin, on the transport of two major resources, C and N.

### Methods

**PLANT PROPAGATION AND EXPERIMENTAL DESIGN**

A single clone of *F. chiloensis* was collected from a natural population on coastal sand dunes at Año Nuevo State Reserve in San Mateo County, California, and propagated through at least six vegetative generations in a greenhouse at the University of Massachusetts at Amherst. For experiments, newly produced ramets were individually rooted while still connected to their parental stolon in pots (11 cm diameter × 11 cm depth) containing fine, acid-washed sand. The stolon was then cut between every other ramet, leaving pairs of connected ramets. Severing stolons has no direct effect on growth in *F. chiloensis* (Alpert 1991). Ramets were 9–12 weeks old during experiments.

Experiments were performed in the same greenhouse that was used for plant propagation. In each experiment (e.g. Figure 1a), a hormone was applied (see below) to the shoot tip (to the surface of the sheath of stipules that covers the apical meristem) of one of the ramets, the ‘target ramet’ in a pair. Labelled C or N was then fed to the other ramet (the ‘source ramet’), and the amount of labelled C or N in the target ramet was measured 2 days later. Each experiment also included a control treatment, which differed from the hormone treatment only in that no hormone was applied to the target ramets (to conserve space, the control treatment for each experiment is not shown in Fig. 1). In all but one experiment (Fig. 1g), the younger ramet in each pair was used as the target ramet, because of the acropetal nature of N sharing in *F. chiloensis* (Alpert 1996). In each experiment, pairs of ramets were randomly assigned to treatments, and treatments were randomly assigned to positions on a single greenhouse bench.

The seven experiments, performed sequentially, were designed to test whether: (i) auxin can increase C import by a ramet when connected ramets are in microsites with similar resource availabilities (Fig. 1a);
Hormonal modification of clonal integration

Fig. 1. Schemes for seven experiments to test effects of auxin (IAA) and cytokinin (BA) on carbon and nitrogen transport between pairs of ramets of Fragaria chiloensis under different conditions of resource availability. Only the hormone treatments are shown; in each experiment there was also a control treatment in which no IAA or BA was applied to the target ramet. The older ramet of the pair is always shown on the left. The target ramet of the pair is on the right in (a–f), and on the left in (g). Numerals on pots refer to N treatment (mg N l–1 watering solution).

(ii) auxin can increase C import by a ramet in a relatively shaded microsite (Fig. 1b); (iii) auxin can increase N import by a ramet when connected ramets are in microsites with similar resource availabilities (Fig. 1c); (iv) auxin can increase N import by a ramet in a microsite with relatively low N availability (Fig. 1d); (v) cytokinin can increase C import when connected ramets are in microsites with similar resource availabilities (Fig. 1e); (vi) cytokinin can increase N import by a ramet in a microsite with relatively low N availability (Fig. 1f); and (vii) auxin in combination with low N availability can increase N transport from a younger to an older ramet, against the usual direction of N transport in F. chiloensis (Fig. 1g).

In the first experiment with auxin (Fig. 1a) and the two experiments with cytokinin (Fig. 1e,f), the time course of the effect of hormone treatment on resource import was tested by including three time treatments (not shown in Fig. 1) in which ramets were labelled, respectively, 24, 48 and 96 h after the start of hormone application. Hormone treatments were replicated four times at each time treatment. No effect was detected in any 24 or 48 h treatments, so only the 96 h treatment was used in the other experiments, and only this treatment is presented in the Results. Hormone treatments in the experiments with one time treatment were replicated six to eight times. Plants were always harvested 48 h after labelling.

RESOURCE TREATMENTS AND HORMONE APPLICATIONS

In one experiment (Fig. 1b), light availability to the target ramets was reduced to 20% of ambient light by shading them with spectrally neutral agricultural shade cloth. This was intended to approximate the mean percentage reduction of ambient light experienced by ramets of F. chiloensis that grow under shrubs of Lupinus arboreus Sims, a dominant, N-fixing shrub at the collection site (Alpert & Mooney 1996). In all experiments, soil N availability was controlled by watering plants with modified Hoagland’s solution (Alpert & Mooney 1986) containing 0, 10 or 15 mg N-NO3 l–1. The highest N concentration was chosen to approximate soil N availability under L. arboreus (Alpert & Mooney 1996). In the three experiments where effect of nutrient patchiness was tested (Fig. 1d,f,g), the source ramet was given 15 mg N-NO3 l–1 and the target ramet was given 0 mg N-NO3 l–1. In the experiments to test C sharing (Fig. 1a,b,e), both ramets were given 10 mg N-NO3 l–1 to eliminate N patchiness as a factor. In the experiment to test the effects of auxin on N sharing in a uniform environment (Fig. 1c), both ramets were given 15 mg N-NO3 l–1, to eliminate patchiness and minimize possible limitation of growth by N.

Applications of auxin followed a non-invasive procedure that increases internal auxin concentration in F. chiloensis (I. A. Tamas, personal communication). Every hour from 09:00 to 18:00 (10 times) on two successive days, 10 µl of 20 μM indole-3-acetic acid (IAA) in Tween MES buffer (Sigma, St Louis, Missouri) was applied externally with a microsyringe to the tip of the shoot (formed by the apical meristem and the covering, rolled stipules of unexpanded leaves). Applications of cytokinin followed the same protocol, except that 100 µM 6-benzylaminopurine (BA) was used instead of auxin. This amount of cytokinin was chosen because preliminary trials showed that about 80 µM was sufficient to produce a plant response, namely shoot elongation after 10 days. Target ramets in the control treatments received applications of buffer without added hormones. To prepare buffer, 1·07 g MES (to give a concentration of 50 mM) and 0·2 ml Tween 80 were dissolved in 100 ml distilled water, adjusted to pH 5·5 with KOH.

CARBON AND NITROGEN LABELLING AND MEASUREMENTS

To label a source ramet with C, the youngest fully expanded leaf of the plant was exposed to 14CO2 for 2 h...
inside a clear polyvinylchloride gas sampling bag at an irradiance of 300 μmol m⁻² s⁻¹. The ¹⁴CO₂ was generated by adding excess 1 M acetic acid to a solution of NaH¹³CO₃ containing 10 μCi in a vial inside the bag. The connected target ramet was either given the same irradiance or, for the experiment that included light patchiness (Fig. 1b), shaded during exposure. Plants were then returned to the greenhouse for 48 h. At the end of this time, each ramet was separated into roots, stem plus unexpanded leaves, expanded leaf blades, and their petioles, to prevent further movement of label between ramets and their parts. Parts were arranged on a sheet of cardboard and dried in a press.

In the first experiment with auxin (Fig. 1a) and the experiment with cytokinin (Fig. 1e), ¹⁴C labelling was initially measured using autoradiography. A sheet of X-ray film (Kodak X-OMAT AR) was placed on top of a pressed plant, exposed for 12 days at −70 °C, and developed with a Konica X-ray film processor (QX-60A). Developed films were scanned into Adobe Photoshop 5, and labelling in each plant part was classified as heavy, light or undetectable, based on the average grey-scale value of the part as measured by the software.

As autoradiographs indicated that ¹⁴C-labelling was heaviest in the roots and the stem-tip region, stem tips were used for measurements of labelling via scintillation counting. This was done for the target ramets in the hormone and control treatments in all the experiments on C sharing (Fig. 1a,b,e). Each tip was weighed, frozen in liquid N₂, and ground with a mortar and pestle. A subsample of about 20 mg of the powder was weighed, placed in a scintillation vial containing 6 ml scintillation liquid (EconoSafe, RPI, Mount Prospect, IL), and counted for ¹⁴C activity in a Beckmann LS 6000 SL scintillation counter. Activity (CPM) was expressed on a per tip basis by multiplying the count, after correction for background levels measured in ramets from unlabelled pairs, by subsample mass/total tip mass.

To label a source ramet with N, 75 ml modified Hoagland’s solution containing 15 mg N-Na¹⁵NO₃, I⁻¹ (99 atom %; Icon Services, Summit, NJ) was added to the soil around the plant 96 h after the start of hormone application. Source ramets in control treatments received the same amount of solution, prepared with unlabelled N. Stem tips of the target ramets were ground and sent for isotope ratio mass spectrometry to the Stable Isotope Ratio Mass Spectrometer Laboratory in the Department of Earth and Atmospheric Sciences at the University of Albany, or to the Stable Isotope/Soil Biology Laboratory in the Institute of Ecology at the University of Georgia. The amount of labelling was expressed as excess atom % (proportion of N present as ¹⁵N in excess of the proportion measured in ramets from unlabelled pairs, ×1000).

To provide a non-destructive measure of whether hormone applications affected plant growth, the length from the tip of the stem to the base of the youngest petiole of each ramet (referred to as ‘stem tip length’) was measured at the start of hormone application and again at harvest. Growth was expressed on a relative basis as final length/starting length.

**DATA ANALYSIS**

As sample sizes were small and some distributions appeared non-normal, non-parametric statistics (Fisher’s exact test; Siegel & Castellan 1988) were used to test for treatment effect (control versus hormone) on amounts of labelled C or N found in the target ramets. For each experiment, the values of ¹⁴C or ¹⁵N in the target plants were classed as high (in the upper 50% of the values) or low (in the lower 50%), then tallied by class × treatment in a 2 × 2 table. A parallel set of one-way ANOVA’s, run in SYSTAT 9.0, gave similar results. To test for the effect of hormones on growth, plant growth data from the experiments with a given hormone were pooled and a one-way ANOVA was run on the arcsines of the square roots of the values.

**Results**

**EFFECTS OF HORMONE APPLICATIONS ON LABELLING OF TARGET RAMETS**

Effect of hormone treatment on resource import in each experiment (Fig. 1a–g) was tested by comparing the amounts of labelled resource fed to the source ramets that were found in the target ramets in the control and hormone treatments (Fig. 2a–g). For example, in the first experiment, ¹⁴C-radioactivity in the targets to which no hormone was applied (Fig. 2a, bar labelled ‘Control’) was compared to ¹⁴C-radioactivity in the targets to which IAA was applied (Fig. 2a, bar labelled ‘IAA’).

When target and source ramets were given similar resource availabilities, the mean amount of ¹⁴C radioactivity measured in shoot apices did not differ significantly between control and IAA-treated target ramets (Fig. 2a). However, the amount of labelling varied greatly among replicates in the IAA treatment, as reflected by the large SEM for this treatment. Similar variability was observed in the autoradiographs of the IAA-treated target plants from this experiment (Table 1, Experiment 1). Two replicates showed heavy labelling, one showed only light labelling, and one showed none. By comparison, none of the control ramets showed heavy labelling, two showed light labelling, and two showed no detectable labelling. Together, scintillation counts and autoradiography suggested that IAA treatment increased import in some but not all replicates. These data also indicated that ramets sometimes imported at least small amounts of C from connected ramets, even without hormone treatment.

Auxin more consistently increased C-labelling of shaded ramets (Fig. 2b). The mean amount of ¹⁴C detected in IAA-treated target ramets was about double
Effects of hormone applications on labelling of shoot apices of target ramets for each part: ++, heavy labelling; +, light labelling; –, no detectable labelling. From four replicate targets, with the replicates in each treatment listed in the same order for each plant part in each treatment in each experiment, the four symbols show results.

Table 1. $^{14}$C-labelling of organs of target ramets in two experiments, based on grey-scale levels in scanned autoradiographs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Roots</th>
<th>Stem</th>
<th>Leaf blades</th>
<th>Petioles</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td>+, +, –</td>
<td>+, +, –</td>
<td>+, +, –</td>
<td>+, –, –</td>
</tr>
<tr>
<td>+IAA:</td>
<td>++, +, +, –</td>
<td>++, +, +, –</td>
<td>++, +, +, –</td>
<td>+, –, –</td>
</tr>
<tr>
<td><strong>Experiment 5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control:</td>
<td>+, +, –</td>
<td>+, –, –</td>
<td>+, +, +</td>
<td>–, –, –</td>
</tr>
<tr>
<td>+BA:</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
<td>+, +, +</td>
</tr>
</tbody>
</table>

*Experiment 1, effect of IAA on carbon import under uniform conditions; Experiment 5, effect of BA on carbon import under uniform conditions.

For each plant part in each treatment in each experiment, the four symbols show results from four replicate targets, with the replicates in each treatment listed in the same order for each part: ++, heavy labelling; +, light labelling; –, no detectable labelling.

Plant growth

Hormone applications did not significantly affect the relative increase in stem tip length (see definition in Methods) of ramets during the period between the start of hormone application and plant harvest, although there was a nominal tendency for more growth in hormone-treated than in control targets. Stem tip lengths of the hormone-treated and control target ramets increased by 48 and 36%, respectively, in the experiments with auxin ($P$ [ANOVA] = 0.1); and by 56 and 53%, respectively, in the experiments with cytokinin ($P$ = 0.8).

Discussion

The results support the hypothesis that hormones can modify resource sharing between connected ramets in clonal plants. Patterns of $^{14}$C- and $^{15}$N-labelling of target ramets show that one of the two hormones tested, IAA, significantly and strongly increased the import of C and N by ramets of $F$. chiloensis that were located in relatively light-poor (Fig. 1b; Fig. 2b) or N-poor (Fig. 1d; Fig. 2d) microsites. The other hormone, BA, did not significantly increase mean $^{15}$N-labelling of target ramets located in relatively N-poor microsites (Fig. 1f; Fig. 2f). The answer to our second question, whether hormones can increase resource import by ramets that experience relatively low resource availability, is yes. We conclude that $F$. chiloensis could use auxin to modulate the degree of resource sharing induced by environmental heterogeneity.

On the other hand, we found mixed evidence as to whether elevated hormone concentrations can increase...
resource import when ramets experience similar resource availabilities. As measured by scintillation counting, there was no significant difference between mean import of C or N by auxin- or cytokinin-treated and control target ramets into shoot tips when targets and sources were given similar resource treatments (Fig. 1a,c,e; Fig. 2a,c,e). On the other hand, autoradiography (Table 1) suggests that at least some auxin- and cytokinin-treated target ramets did import more C than the corresponding controls when ramets were given similar resource treatments. One possibility is that spatial differences in ambient light availability in the greenhouse were large enough to enable hormonal effects in some replicates in these experiments. Another possibility is that hormones affected import by some plant parts other than shoot tips. The answer to our first question, whether hormones can increase resource sharing in the absence of resource patchiness, is uncertain.

There was no evidence that auxin could increase N transport from younger to older ramets (Fig. 1g, Fig. 2g). The answer to our third question, whether hormones are responsible for directionality of nutrient transport, is no.

Two alternative models have been proposed for the relationship between modules of the same type within plants (Henriksson 2001). The first model views such modules as competitors for resources (Sachs et al. 1993). This model is supported mostly by evidence from effects of shading different branches within a ramet: shading individual branches of non-clonal trees or herbs often reduces their import of water and nutrients (Henriksson 2001; Honkanen & Haukioja 1994; Sachs & Novoplansky 1997). Possible mechanisms for competition between modules include increases in conductance (Whitehead et al. 1996) or in hormone synthesis (Sachs & Novoplansky 1997) in modules given greater access to resources.

The second model views modules as co-operators for resources. This model is supported mostly by evidence from effects of reducing resource availability to different ramets within a clone: reducing light, water or soil nutrient availability to individual ramets of clonal plants often increases their import of C, water or nutrients (Hutchings & Wijesinghe 1997; Jonsdóttir & Watson 1997; Lötscher & Hay 1996). Possible mechanisms for co-operation between modules include gradient-driven resource translocation.

Under different environmental conditions, F. chiloensis can show either competition or co-operation between ramets for resources (Alpert 1996; Alpert 1999). This suggests that, at least at the level of ramets in some clonal species, these two models are really alternative possible states: clones can use environmental cues to switch between competition and co-operation among ramets, and either concentrate resources in particular ramets or equalize resources among ramets. Gradient-driven resource translocation plus hormonal modification could provide a mechanism for this in F. chiloensis.

This raises several interesting research questions. On the physiological side, how do auxin concentrations within ramets respond to external resource availabilities? Does high resource availability induce auxin synthesis, leading to ramet competition; or does low resource availability induce synthesis, leading to co-operation or perhaps to ‘over-sharing’ (Alpert 1999). For branches within shoots of non-clonal plants, there is only evidence that high resource availability can induce auxin synthesis (Tsxi Sachs, personal communication), but little is known about the effects of differential resource availability on hormone concentrations in connected ramets in clones. Do hormones modify resource import by promoting or inhibiting growth and changing resource demand, or by stimulating vascular differentiation that lowers resistance to transport (Jacobs 1998; Sachs & Novoplansky 1997)? We saw no significant effect of hormone treatments on the growth in length of shoot tips of target ramets, which is more consistent with the second possibility. However, hormones could have affected other aspects of growth, such as growth of roots or of source ramets.

On the evolutionary side, under what environmental conditions might ramet competition increase clonal fitness more than ramet co-operation? It could be advantageous for F. chiloensis on dunes to concentrate growth in shaded ramets, if they are also likely to be located under shrubs where soil N is relatively high. In habitats where resource availabilities to any one ramet are too low to allow it to reproduce, it could be advantageous for a clone to concentrate resources in a few ramets and thereby achieve some reproduction.

Our findings provide initial evidence that hormones, already known to regulate sink strength within non-clonal plants (Kuiper 1993), can also modify resource sharing between ramets within clones. Hormonal control is a potential mechanism by which clones might concentrate resources in particular ramets. Hormones may be an important mechanism by which clonal plants integrate the physiology and growth of connected ramets.

Acknowledgements

We thank Imre A. Tamas at Ithaca College for sharing his auxin application protocol; Hadas H. Parag for technical advice and research assistance; Dirk Enters, Bree Goldstein, Don Medd, Meaghan Shaffer and Madeeha Yousef for additional research assistance; Tsxi Sachs for technical advice and comments on the manuscript; Ronald Beckwith and Monika Johnson for greenhouse maintenance; Alice Cheung, Dan Hebert, Sandra Peterson, and Anne Simon for use of facilities in their labs; Michael Marcotrigiano and Bernard Rubinstein for additional technical advice; Steven Howe and Tom Maddox for the mass spectrophotometry; and the California Department of Parks and Recreation for permission to collect plants at Año Nuevo State Reserve. Research was supported by US
References


Received 16 July 2001; revised 1 October 2001; accepted 14 October 2001