Conflict and Cooperation in the Tropical Wasp, *Parachartergus colobopterus*, and the Chimeric Multicellular Organism, *Dictyostelium discoideum*

by

Thomas Gene Platt

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

Master of Arts

APPROVED, THESIS COMMITTEE:

Joan E. Strassmann, Professor, Co-chair
Ecology and Evolutionary Biology

David C. Queller, Professor, Co-chair
Ecology and Evolutionary Biology

Richard H. Gomer, Professor
Biochemistry and Cell Biology

HOUSTON, TEXAS

MAY 2003
ABSTRACT

Conflict and cooperation in the tropical wasp, *Parachartergus colobopterus*, and the social amoeba, *Dictyostelium discoideum*

by

Thomas Gene Platt

Several transitions to higher levels of biological organization have punctuated the history of life. These transitions occur when cooperative alliances lead to the integration of non-identical partners into more complex wholes. Yet there is inevitable conflict within these cooperative alliances. In the following chapters I investigate reproductive conflicts of interest between cooperators within insect societies and chimeric multicellular organisms. In the first chapter I show that in the tropical wasp, *Parachartergus colobopterus*, workers use aggression toward totipotent, emerging females to influence the reproductive future of the latter. By doing this workers resolve conflict over who reproduces in accord with their collective interests. In the second chapter I show that environmental heterogeneity can affect the outcome of conflict between co-aggregating clonal lineages of *Dictyostelium discoideum*. This helps account for the coexistence of cheaters and victims in natural populations of *D. discoideum*. 
ACKNOWLEDGMENTS

Many people have been critically important to my intellectual development over the past five years. Above all others I would like to thank Joan Strassmann and Dave Queller for all around, exceptional advising. Without them this work would not have been possible. I’d also like to thank Kevin Foster for a lot of guidance and for being an excellent partner on all of our slime mold projects.

More generally I’d like to thank our supportive lab group and department. I thank Natasha Mehdiabadi, Wendy Castle, Mike Henshaw, Eva Toth, Dirk Jan Ronhaar, Lorenzo Santorelli, Jeff Smith, and Angelo Fortunato for helpful discussions and support in the laboratory. I also thank the people at Mountain Lake Biological Station for support in the field. Within the department, big thanks to Barryy Sullender for always being willing to challenge me in discussion on any topic. Similarly thanks to Evan Siemann, Lisa Meffert, Bill Rogers, and Dr. Harcombe for regularly challenging me.

I am grateful to Scott Baggett for assistance in developing the statistical models used in analysis of the wasp data. Also thanks to Evan Siemann, and Lisa Meffert for statistical advice. I thank the Universidad Central de Venezuela, Facultad de Agronomía, in Maracay, for allowing us to work on their campus for the wasp study.

This work was supported by US National Science Foundation grants IBN-9507515, IBN-9808809, DEB-0075581 and DEB-0108478 to JES and DCQ and by a Wray-Todd Fellowship and a Mountain Lake Biological Station Predoctoral Research Fellowship to TGP.
TABLE OF CONTENTS

Chapter 1. Aggression and worker control of caste fate in a multiple queen wasp, Parachartergus colobopterus.............................Page 1

  Introduction.................................................................Page 2

  Methods...........................................................................Page 4

  Results.............................................................................Page 12

  Discussion........................................................................Page 18

Chapter 2. Environmental heterogeneity and the coexistence of cheaters and victims in the social amoeba Dictyostelium discoideum .................................................................Page 24

  Introduction.......................................................................Page 25

  Methods............................................................................Page 27

  Results.............................................................................Page 32

  Discussion........................................................................Page 44

References...........................................................................Page 48
LIST OF TABLES

Table 1. Colony characteristics of 34 Parachartergus colobopterus nests..........................................................Pages 6-7

Table 2. Poisson Regression for colony and behavioural class effects on distribution of males, mated females, and ovarian development.......................................................Page 13

Table 3. Ordinal Logistic Regression for colony and behavioural class effects on age.................................................Page 14

Table 4. Description of different environmental conditions.................................................................Page 30

Table 5. Overall effect of environment on the change in each strain’s proportions between the initial cell suspension and the spore population for each pair of competing clones..............Page 33

Table 6. Influence of environment and the identity of the clone pair on the average absolute magnitude of changes in clone proportions..........................................................Page 43
LIST OF FIGURES

Figure 1. Proportion of victims, aggressors, and randomly
sampled females with ovarian development.................................Page 13

Figure 2. Percent histogram showing the percentage of mated
victims, mated aggressors, and mated females having a total
number of mature and nearly mature eggs.................................Page 17

Figure 3. Average change, across all environments, in the
proportion of the first clone between the initial cell suspension
and the spore population for each of the competitions.......................Page 34

Figure 4. Average change in the proportion of strain V55C2
when in competition with strain V326D1 in 8 different
environmental contexts..................................................................Page 35

Figure 5. Average change in the proportion of strain V342B2
when in competition with strain V301B2 in 8 different
environmental contexts..................................................................Page 36

Figure 6. Average change in the proportion of strain V324B1
when in competition with strain V55C2 in 8 different
environmental contexts..................................................................Page 37
Figure 7. Average change in the proportion of strain V324B1 when in competition with strain V326D1 in 8 different environmental contexts.................................................................Page 38

Figure 8. Average change in the proportion of strain V327A1 when in competition with strain V336B1 in 8 different environmental contexts.................................................................Page 39

Figure 9. Average change in the proportion of strain V336B1 when in competition with strain V77B in 8 different environmental contexts.................................................................Page 40

Figure 10. Average change in the proportion of strain V301B2 when in competition with strain V56A2 in 8 different environmental contexts.................................................................Page 41

Figure 11. Average change in the proportion of strain V327A1 when in competition with strain V77B in 8 different environmental contexts.................................................................Page 42

Figure 12. Average magnitude, across all 8 mixes, of changes in clone proportions during development for each of the 8 environments.................................................................Page 43
CHAPTER 1

Aggression and worker control of caste fate in a multiple queen wasp, *Parachartergus colobopterus*

ABSTRACT

Though famously cooperative, social insect colonies harbour considerable potential for genetic conflict among colonymates. This conflict may be expressed behaviourally as aggression by workers. We investigated aggression in 34 colonies of the wasp, *Parachartergus colobopterus*, by evaluating the characteristics of both instigators and victims of aggressive interactions. We estimated genetic relatedness and queen number using DNA microsatellites and found that that workers and emerging females should be most in conflict over the caste of the latter when there are many queens on the nest. We found that aggressive interactions are more likely to involve older workers attacking either males or younger workers, and that victim and aggressor females have more ovarian development than randomly-sampled colonymates. Moreover, mated females with low levels of ovarian development relative to active queens were also more likely to be aggressors and victims than randomly sampled females. Aggression among females supports the hypothesis that older workers use aggression toward younger females as a means of policing the development of emerging females into queens. Also workers may use aggression to suppress the reproduction of some mated females. Our findings thus support the hypothesis that genetic conflicts of interest motivate worker aggression in swarm-founding wasp colonies.
INTRODUCTION

Insect societies typically exhibit high degrees of cooperation with a reproductive division of labour so great that they are often viewed as superorganisms (Seeley 1989; Wilson & Sober 1989; Moritz & Southwick 1992; Ratnieks & Reeve 1992). Kin selection theory has served as a framework providing insight into the evolution of this cooperation (Hamilton 1964; Bourke & Franks 1995; Crozier & Pamilo 1996; Bourke 1997; Queller & Strassmann 1998). It has also provided a framework for understanding potential conflicts within these societies since colonymates are genetically distinct from one another and thus have different genetic interests concerning reproduction (Hamilton 1964; Trivers & Hare 1976; Ratnieks 1988; Pamilo 1991; Ratnieks & Reeve 1992; Queller & Strassmann 1998; Keller & Chapuisat 1999; Keller & Reeve 1999). Conflicts of interest between colonymates can manifest themselves in a variety of ways including oophagy and direct aggression (e.g. Ratnieks & Visscher 1989; Gobin et al. 1999). Our study focuses on whether genetic conflicts of interest motivate behavioural aggression in the well-studied swarm-founding neotropical wasp, *Parachartergus colobopterus* of the tribe Epiponini (Strassmann et al. 1991, 1997, 2002; Goodnight et al. 1996; Herman et al. 2000).

Epiponine wasp societies have many complex features: they have an advanced division of labour, task partitioning, alarm and trail pheromones, and large colony sizes (Jeanne 1980; Jeanne 1991; Zucchi et al. 1995; Jeanne, in press). Yet, in contrast with other highly eusocial insects, many epiponine wasps have weak caste differentiation (Bourke 1999; O’Donnell 1998; Jeanne, in press). *P. colobopterus* colonies typically have large numbers of workers and a varying number of singly-mated queens, with little
or no morphological caste differences between workers and queens (Strassmann et al. 1991, 1997, 1998; Goodnight et al. 1996). As in some other epiponine societies (Metapolybia azecoides, Synoeca surinama, West-Eberhard 1978, 1981), emerging P. colobopterus females are totipotent. They can become either workers or queens (Strassmann et. al. 2002). This leads to potential conflict over caste determination because each individual gains more from her becoming a queen than her colonymates gain (Strassmann 1989; Bourke & Ratnieks 1999; Ratnieks 2001, Reuter & Keller 2001).

In accord with worker collective interests, new P. colobopterus queens are produced only when queen number is low (Strassmann et al. 1991, 2002; Queller et al. 1993). This raises the question of how totipotent females emerging on nests with many queens are kept from themselves becoming queens when it may be in their interest to do so. Females attempting to become a queen could be forced to become workers instead (West-Eberhard 1978, 1981; Herman et al. 2000; Strassmann et al. 2002), an option not possible when there are fixed caste differences, as in Melipona stingless bees, where workers slaughter excess queens (Imperatriz-Fonseca & Zucchi 1995).

Eusocial wasp males seldom work or forage; yet they do depend on the colony for support. Consequently, the presence of males on a nest can impose substantial costs to social insect colonies (O’Donnell 1999). Moreover, workers are more related to female larvae than to adult males, and thus should favour investing in the former over the latter (Hamilton 1972; Trivers and Hare, 1976; Starks & Poe 1997). Workers may use aggression toward males to minimize the colony costs they impose and discourage investment in males.
This study examines aggression on a large sample of *P. colobopterus* colonies. We predict that males and young females will be the targets of aggression because of genetic conflicts with workers.

**METHODS**

*Sampling*

We observed 34 *P. colobopterus* colonies on the campus of the Universidad Central de Venezuela at Maracay, Venezuela (10° 16' N 67° 36' W, altitude 445 m) 6-10, August 1999, during the rainy season. To be able to sample and observe the entire nest, we removed the surrounding envelope of each colony. This does not disturb the nest combs because the envelope and the combs are independently attached to the substrate. This nest structure means we can observe all areas of the nest for activity, and can sample wasps from any part of the nest. Following envelope removal we gave the wasps at least 10 minutes to settle down and resume normal activity before starting observations.

We were interested in quantifying two aspects of aggressive behaviour: frequency of aggression, and who participates in aggressive interactions. To quantify the aggression level of each colony, we counted all aggressive interactions that occurred on the exposed combs during 30 minutes (Table 1). These behavioural observations were taken from 900 to 1600 when the wasps were most active. We did not sample behaviour during rainy spells, when the wasps become less active. Thirty minutes is a sufficient amount of time to characterise aggressive activity on a colony. We base this on hundreds of hours of observing these wasps (Strassmann et al. 1991, 1997, 1998, 2002, Queller et al. 1993; Herman et al. 2000). We also base this on a specific preliminary study from the previous
year in which we watched fewer colonies for periods of three hours or more across days, and noted that 30 minutes was an adequate sample period (data not shown). We also felt that choosing to sample and watch for 30 minutes and thus being able to include 34 colonies in our study was the best strategy for getting at underlying patterns of the role of aggression in reproductive regulation. In 30 minutes we observed between 0 and 57 aggressive acts, averaging 14 (S.D. 13) per colony, an average and variance high enough to detect differences among colonies (Table 1).

An aggressive interaction involved one wasp working its mandibles over another in a chewing, snapping, or biting action, often with the aggressor on top of the victim. Aggressive interactions also often included the aggressor curving her gaster and exposing her sting to the victim. The victim sometimes responded by curling over on her side and remaining motionless, even after we pulled the actor off (Strassmann et al. 1997). Sometimes the aggressor dragged the victim to another part of the nest. Typically each such aggressive interaction lasted several seconds and may be similar to an exaggerated form of biting behaviour of Polybia wasps (O'Donnell & Jeanne 1995; O'Donnell 2001a). Aggressive interactions had a clear beginning and usually would have ended with the victim running away or flying off the nest had we not removed one party. These aggressive acts were quite stereotyped and did not vary greatly in intensity, making them a clear class for sampling. We distinguished aggression from social grooming by noting that the latter involves one wasp slowly and gently working her mouthparts and mandibles over another wasp's body while antennating her and never involves snapping or biting, the exposure of the actor's sting, or the pulling of the recipient to another part
Table 1. Colony characteristics

<table>
<thead>
<tr>
<th>Colony</th>
<th># Combs</th>
<th># Adults</th>
<th>% Cells in the central comb</th>
<th>% Empty Cells</th>
<th>Total Aggressive Acts/ hour</th>
<th>% Aggressive Acts involving females</th>
<th>Unmated Female aggressors</th>
<th>Mated Female Aggressors</th>
<th>Unmated Female Victims</th>
<th>Mated Female Victims</th>
<th>Male Victims</th>
<th>Average Colony Relatedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>97</td>
<td>130</td>
<td>51</td>
<td>0</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>300</td>
<td>323</td>
<td>3</td>
<td>0</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>500</td>
<td>382</td>
<td>13</td>
<td>28</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>395</td>
<td>806</td>
<td>15</td>
<td>8</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>500</td>
<td>466</td>
<td>35</td>
<td>66</td>
<td>86</td>
<td>11</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>520</td>
<td>532</td>
<td>10</td>
<td>12</td>
<td>100</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>65</td>
<td>62</td>
<td>24</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.21</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>110</td>
<td>86</td>
<td>10</td>
<td>6</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>270</td>
<td>235</td>
<td>14</td>
<td>14</td>
<td>100</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>580</td>
<td>312</td>
<td>9</td>
<td>20</td>
<td>80</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0.24</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>130</td>
<td>131</td>
<td>35</td>
<td>18</td>
<td>86</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>160</td>
<td>252</td>
<td>17</td>
<td>38</td>
<td>100</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0.24</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>65</td>
<td>63</td>
<td>5</td>
<td>10</td>
<td>100</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.32</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>225</td>
<td>274</td>
<td>79</td>
<td>50</td>
<td>86</td>
<td>10</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0.10</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>142</td>
<td>109</td>
<td>28</td>
<td>16</td>
<td>100</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0.19</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>360</td>
<td>222</td>
<td>64</td>
<td>42</td>
<td>67</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>0.11</td>
</tr>
<tr>
<td>17</td>
<td>12</td>
<td>600</td>
<td>196</td>
<td>11</td>
<td>0</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.26</td>
</tr>
<tr>
<td>18</td>
<td>20</td>
<td>670</td>
<td>383</td>
<td>19</td>
<td>42</td>
<td>56</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>0.27</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>105</td>
<td>79</td>
<td>9</td>
<td>2</td>
<td>100</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-0.01</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>80</td>
<td>65</td>
<td>5</td>
<td>12</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.17</td>
</tr>
<tr>
<td>21</td>
<td>7</td>
<td>240</td>
<td>134</td>
<td>32</td>
<td>68</td>
<td>67</td>
<td>13</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>9</td>
<td>0.31</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
<td>415</td>
<td>189</td>
<td>13</td>
<td>36</td>
<td>76</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0.18</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>85</td>
<td>59</td>
<td>25</td>
<td>2</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.72</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>60</td>
<td>118</td>
<td>3</td>
<td>0</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.30</td>
</tr>
<tr>
<td>25</td>
<td>13</td>
<td>430</td>
<td>211</td>
<td>15</td>
<td>0</td>
<td>---</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.31</td>
</tr>
<tr>
<td>26</td>
<td>6</td>
<td>280</td>
<td>254</td>
<td>28</td>
<td>30</td>
<td>91</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0.28</td>
</tr>
</tbody>
</table>
Table 1, continued. Colony Characteristics

<table>
<thead>
<tr>
<th>Colony</th>
<th># Combs</th>
<th># Adults</th>
<th># Cells in the central comb</th>
<th>% Empty Cells</th>
<th>Total Aggressive Acts/ hour</th>
<th>% Aggressive Acts involving females</th>
<th>Unmated Female Aggressors</th>
<th>Mated Female Aggressors</th>
<th>Unmated Female Victims</th>
<th>Mated Female Victims</th>
<th>Male Victims</th>
<th>Average Colony Relatedness</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>12</td>
<td>270</td>
<td>171</td>
<td>44</td>
<td>114</td>
<td>81</td>
<td>20</td>
<td>1</td>
<td>12</td>
<td>2</td>
<td>8</td>
<td>0.32</td>
</tr>
<tr>
<td>28</td>
<td>12</td>
<td>160</td>
<td>96</td>
<td>28</td>
<td>36</td>
<td>85</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0.40</td>
</tr>
<tr>
<td>29</td>
<td>17</td>
<td>430</td>
<td>262</td>
<td>13</td>
<td>56</td>
<td>75</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0.14</td>
</tr>
<tr>
<td>30</td>
<td>17</td>
<td>290</td>
<td>266</td>
<td>29</td>
<td>38</td>
<td>73</td>
<td>8</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
<td>0.24</td>
</tr>
<tr>
<td>31</td>
<td>3</td>
<td>110</td>
<td>134</td>
<td>98</td>
<td>32</td>
<td>50</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0.05</td>
</tr>
<tr>
<td>32</td>
<td>5</td>
<td>205</td>
<td>467</td>
<td>92</td>
<td>24</td>
<td>55</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0.07</td>
</tr>
<tr>
<td>33</td>
<td>20</td>
<td>350</td>
<td>536</td>
<td>88</td>
<td>78</td>
<td>78</td>
<td>17</td>
<td>2</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>0.15</td>
</tr>
<tr>
<td>34</td>
<td>21</td>
<td>55</td>
<td>111</td>
<td>77</td>
<td>18</td>
<td>60</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Males were aggressors in only three of the sampled aggressive interactions.
of the nest (Strassmann et al. 1997). Also, social grooming does not induce curling in the
recipient. We distinguished aggression from solicitation for trophylaxis by noting that
the key feature of the latter always involves bites directed toward the mandibles of
another wasp and seldom extends to generalized aggression with bites directed at the rest
of the body (Strassmann et al. 1997).

We collected one participant of each interaction (either the aggressor or the
victim) and labelled it according to its role in the interaction (Table 1). We could not
accurately collect both interactants in a single interaction. This form of collecting means
that we have a very accurate sample of aggressors and of victims, but could not determine
whether or not sub categories of aggressors attacked sub categories of victims. This did
not prove to be a problem because of the strong overall patterns we found (see Results)
and because we were most interested in also comparing victims and aggressors to a
random sample that by definition could not be paired with particular acts. The random
sample consisted of 12 individuals plucked at random from all over the nest combs and
substrate surface behind the nest structure. We used this random sample to estimate
average relatedness and to provide a baseline for ovarian development and mating status.
We stored all individuals and comb parts in 100% ethanol which previous studies had
indicated was sufficient to allow accurate assessment of ovarian development and
insemination status, and also to preserve DNA for microsatellite genotyping (Strassmann
et al. 2002).

For each colony we counted the number of adults on the nest as well as the
number of combs (Table 1). Counts of adults were necessarily approximate because they
were done on active colonies with live wasps. To gauge colony productivity we
inventoried the number of total cells, empty cells, pupae, larvae, and eggs in the middle comb of each nest, which we removed, knowing the wasps would rapidly replace it (Table 1). These measures allowed us to complete this study without destroying a single colony.

Age and Ovarian Measures

To assess age and ovarian development we dissected all 779 collected individuals (aggressors, victims, and random samples). We classified individuals into four relative age categories using the degree of sclerotization of the last sternite (Gastreich et al. 1993). We determined whether or not a female was mated by the presence of sperm in her spermatheca. We considered ovaries to be developed if they contained any detectable oocytes or eggs; we classified any female lacking such ovarian development as a worker and any mated female with such ovarian development as a queen. Twenty-three percent of the dissected females had some ovarian development but only 7% of all dissected females had mature or nearly mature eggs. We measured the length of the longest oocyte or egg in their ovaries. We also recorded the number of mature and nearly mature eggs in their ovaries. We determined the sex of all collected individuals. Our random sample did not have enough mated females to make comparisons with victim and aggressor females. In order to make assess whether mated victims and aggressors had lower levels of ovarian development than the average mated female we made comparisons with mated females from previous collections of P. colobopterus.
**Genotyping**

We stored the adults at –80°C until DNA extraction. We extracted genomic DNA from adults either from the thorax or the abdomen (Strassmann et al. 1996a). To estimate average genetic relatedness in each colony we amplified five microsatellite loci using 10 µl PCR-reactions and $^{35}$S d-ATP to label products (Strassmann et al. 1996a). We attempted to genotype all collected victims and aggressors and either 12 or 5 adults from the random sample, for a total of 479 wasps, at 5 highly variable microsatellite loci: PACO3155, PACO3304, PACO3417, PACO3457, and PACO3107 (Strassmann et al. 1996b). We genotyped 5 random adults on colonies from which we collected no victims or aggressors or from which we collected mostly male victims. We also genotyped 5 random individuals for 3 other colonies with few adults. We ran these PCR products out on 6% polyacrylamide gels and visualized them by exposing the dried gel to Kodak BioMax MR film. We then assessed the size of the products by comparing bands with an M13 sequencing reaction. Genotypes were scored independently by two people and a genotype was scored as missing only after multiple attempts to amplify the locus. Strassmann et al. (1996a) provides more detail on all protocols used.

**Relatedness Estimation**

We estimated average relatedness among colonymates, based on trinucleotide microsatellite genotypes from the five loci listed above, using Relatedness 5.08 (available at http://gsoft.smu.edu/GSoft.html). For relatedness estimations we weighted colonies equally. We used 479 individuals (victims, aggressors, and the random samples) from all
34 colonies to estimate population allele frequencies for relatedness estimates. To obtain standard errors and statistical tests we jackknifed over the 5 loci under the assumption that the pseudovalues are t-distributed with 4 degrees of freedom (Queller & Goodnight 1989). We used relatedness among the randomly sampled adults in each colony to estimate overall average colony relatedness.

Statistical Methods

To determine if victims, aggressors, and randomly sampled wasps differed in ovarian development we modelled the distribution of females with ovarian development among these groups with a Poisson regression. This analysis allows us to evaluate both colony and behavioural class effects on ovarian development. All dissected females were included in the analysis. We scored females with any detectable oocytes, developing eggs, or layable eggs as having ovarian development. The model uses the following log link function to specify a log linear relationship between the number of females expected with ovarian development and two treatments--the colony (n) and the behavioural class (c)--adjusted by the logarithm of the number of females observed (N):

\[ m_{ij} = N_{ij} \exp(\mu + n_i + c_j) \]

Here \( m_{ij} \) is the expected count of females with ovarian development for the \( i \)th colony and \( j \)th behavioural class, where \( i=(\text{colonies 1, 2, 3, ..., 34}) \) and \( j=(\text{victims, randoms, or aggressors}) \), \( \mu \) is the overall mean of the logarithm of the proportion \( m_{ij}/N_{ij} \), \( n_i \) is the \( i \)th treatment level for the nest treatment, and \( c_j \) is the \( j \)th treatment level for the behavioural class treatment level (Agresti 1990). We evaluated the differences between estimated treatment means using contrasts. We used the same modelling technique to evaluate
whether or not males and mated females had a higher probability of being victims or aggressors than had all females (mated and unmated) or unmated females, respectively.

To test how victims and aggressors differed in age we used an ordinal logistic regression, modelling the probabilities of victims, aggressors, and randomly sampled females belonging to each age class. This model takes into account the colony effects. Because the age classes are ordinal we used the cumulative logit model described below:

\[ p_{1ij} + p_{2ij} + p_{3ij} + p_{4ij} = 1 \]

\[ \logit p_{1ij} = \log \left( \frac{p_{1ij}}{1-p_{1ij}} \right) = \mu_1 + n_i + c_j \]

\[ \logit (p_{1ij} + p_{2ij}) = \log \left( \frac{(p_{1ij} + p_{2ij})}{(1-p_{1ij} - p_{2ij})} \right) = \mu_2 + n_i + c_j \]

\[ \logit (p_{1ij} + p_{2ij} + p_{3ij}) = \log \left( \frac{(p_{1ij} + p_{2ij} + p_{3ij})}{(1-p_{1ij} - p_{2ij} - p_{3ij})} \right) = \mu_3 + n_i + c \]

Here \( p_{Xij} \) is the probability of the \( X \)th response for the \( i \)th colony and the \( j \)th behavioural class, \( \mu X \) is the overall mean of the logit of the \( X \)th response, \( n_i \) is the estimated treatment effect of the \( i \)th colony, and \( c_j \) is the estimated treatment effect of the \( j \)th behavioural class with \( i=(1-34) \) and \( j=( \text{victims, randoms, and aggressors}) \) and \( X=( \text{age classes 1-4}) \) where 1 is the oldest and 4 is the youngest age class (Agresti 1990).

RESULTS

*Age and Ovarian Development*

On average, across all colonies, a greater proportion of victims and aggressors had ovarian development than randomly sampled females had (Fig. 1; Table 2). Victims were 2.35 times more likely to have ovarian development than were randomly sampled females (95% CI: 1.54-3.60). Similarly, aggressors were 1.99 times more likely to have
ovarian development than randomly sampled females (95% CI: 1.33-2.99). We found no significant differences between the aggressors and the victims with respect to ovarian development (Table 2).

Figure 1. Proportion of victims, aggressors, and randomly sampled females with ovarian development (average± S.E., N = 34 colonies). We scored any female with any detectable oocytes or eggs as having ovarian development. The percentage with ovarian development for each behavioural class was calculated by averaging across all colonies.

![Graph showing proportion of females with ovarian development across different behavioral classes](image)

**Table 2. Poisson Regression for colony and behavioural class effects on distribution of males, mated females, and ovarian development**

<table>
<thead>
<tr>
<th>Group</th>
<th>Contrast</th>
<th>Contrast Estimates ± SE</th>
<th>Chi-Square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian Development</td>
<td>Victims - Randoms</td>
<td>0.86 ± 0.22</td>
<td>15.62</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Aggressors - Randoms</td>
<td>0.69 ± 0.21</td>
<td>11.09</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Victims - Aggressors</td>
<td>0.17 ± 0.21</td>
<td>0.63</td>
<td>P=0.4291</td>
</tr>
<tr>
<td>Mated Females</td>
<td>Victims - Randoms</td>
<td>2.21 ± 0.50</td>
<td>19.91</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Aggressors - Randoms</td>
<td>1.89 ± 0.49</td>
<td>14.97</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Victims - Aggressors</td>
<td>0.32 ± 0.35</td>
<td>0.85</td>
<td>P=0.3576</td>
</tr>
<tr>
<td>Males</td>
<td>Victims - Randoms</td>
<td>1.98 ± 0.28</td>
<td>51.14</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Aggressors - Randoms</td>
<td>0.24 ± 0.38</td>
<td>0.42</td>
<td>P=0.5173</td>
</tr>
<tr>
<td></td>
<td>Victims - Aggressors</td>
<td>1.74 ± 0.30</td>
<td>33.44</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Analyses based on all wasps collected that belong to the corresponding group.
Victims were significantly younger than random females and aggressors were significantly older than random females (Table 3). Victims were 1.68 times more likely than randomly sampled females to belong to the 2 youngest age classes (95% Wald CI: 1.10-2.54). In contrast, aggressors were 0.57 times less likely to belong to the 2 youngest age classes than were randomly sampled females (95% Wald CI: 0.40-0.81).

**Table 3. Ordinal Logistic Regression for colony and behavioural class effects on age**

<table>
<thead>
<tr>
<th>Behavioural class</th>
<th>Number of colonies</th>
<th>Number of wasps</th>
<th>Maximum Likelihood Estimate ± SE (Randoms as baseline)</th>
<th>Chi-Square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Victims</td>
<td>27</td>
<td>189</td>
<td>0.53 ± 0.13</td>
<td>15.52</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Aggressors</td>
<td>29</td>
<td>184</td>
<td>-0.55 ± 0.12</td>
<td>20.95</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Analysis based on all collected aggressors and victims

*The Presence of Males*

Colonies from which we collected males had higher proportions of empty comb cells (one-tailed Mann-Whitney U test: U=41.5, N₁=N₂=17, P<0.001) and lower proportions of comb cells containing eggs (one-tailed Mann-Whitney U test: U=67, N₁=N₂=17, P<0.01), pupae (one-tailed Mann-Whitney U test: U=83, N₁=N₂=17, P<0.05), and larvae (one-tailed Mann-Whitney U test: U=94, N₁=N₂=17, P<0.05) than colonies without males. For each of these analyses one-tailed tests are appropriate because we predict that the presence of males should cause productivity costs.

Aggression levels involving only females were higher in colonies with males (Mann-Whitney U test: U=54, N₁=N₂=17, P<0.01). Males were more likely to be observed as victims than as either randomly sampled individuals or as aggressors (Table 2). Males were 7.28 times more likely to be a victim than to be randomly sampled (95% CI: 4.23-12.55). However males were not more likely to be aggressors than to be
randomly sampled (Table 2). Males were observed as aggressors in only three of 184 aggressive interactions, cases where male food solicitation escalated into outright aggression.

*Mated females and Aggression*

Mated females comprised 8.8% of all victims and aggressors collected. The ovarian development of these mated victims and aggressors is consistent with that of either young developing queens or queens with regressed ovaries, but not with that of mature, reproductively active queens. To ascertain this, we compared the ovarian development of our mated victims and aggressors with that of 58 mated females from 22 colonies dissected from previous collections of *P. colobopterus*. Sixty-seven percent of the mated victims and 53% of mated aggressors did not have any eggs in their ovaries. The same was true for only 14% of the mated females from a previous collection (Henshaw et al. 2000). Moreover, mated victims and aggressors had significantly fewer mature or nearly mature eggs in their ovaries than these wasps had (Kruskal-Wallis test: $H_2=45.77, P<0.0001$; Fig. 2). By contrast, reproductively active *P. colobopterus* queens have substantial numbers of mature or nearly mature eggs in their ovaries ($\bar{X} \pm SD=36.6\pm14.1$ eggs, $N=8$, range 4-50; data from Strassmann et al. 2002). The mated females observed in aggressive interactions had considerably fewer mature and nearly mature eggs in their ovaries ($\bar{X} \pm SD=2.1\pm2.0$ eggs, $N=34$, range 0-9). Thus, the ovarian development of mated victims and aggressors is consistent with that of mated females with poorly developed ovaries. These results are consistent with the hypothesis that the
reproductive activity of these mated females had been suppressed by workers forcing them to function as workers (West-Eberhard 1978, 1981; Herman et al. 2000). Mated females were 9.13 times more likely to be victims (95% CI: 3.46-24.12) and 6.59 times more likely to be aggressors (95% CI: 2.54-17.14) than randomly sampled females. However mated females were not more likely to be aggressors than victims (Table 2).

Relatedness and Aggression

Higher proportions of randomly sampled young females had ovarian development on colonies with higher relatedness, as compared to those on colonies with lower average colony relatedness (one-tailed Spearman rank correlation: $r_s=0.35$, $N=32$, $P<0.05$). For this analysis we excluded two colonies for which we randomly sampled only one female of the younger age classes. We found no significant correlation between average colony relatedness and the frequency of per capita aggression involving only females (one-tailed Spearman rank correlation: $r_s=0.04$, $N=34$, $P=0.4144$). One-tailed tests are appropriate for these analyses because we predict that lower relatedness should lead proportionally more females to develop their ovaries leading to higher levels of aggression from other females.
Figure 2. Percent histogram showing the percentage of mated victims, mated aggressors, and mated females having a total number of mature and nearly mature eggs in a three-egg interval. All mated females involved in aggressive interactions (as either aggressor or victim) had few mature and nearly mature eggs in their ovaries. In contrast a sample of 58 mated females from 22 different colonies included females with few eggs as well as females with considerably more eggs. This indicates that the mated aggressors and victims have levels of ovarian development consistent with poorly developed mated females.
DISCUSSION

The main results of this study support the hypothesis that genetic conflicts of interest among colony members motivate aggression by workers towards young females attempting to reproduce. Workers may attack females to suppress them from reproducing when there are already queens in the colony (Strassmann et al. 2002).

Aggression involving males

In colonies where males are present, they are often victims of aggression. The most likely explanation for this aggression is that it serves to drive males from the colony, as has been reported for many other social wasps (e.g. Evans and Eberhard 1970 p. 150). This would limit further investment in males and reduce further productivity costs on the colony.

Conflict involving mated females

Overall there was much more aggression involving unmated females than mated females. However, we found that mated females are disproportionately both the targets and the instigators of aggressive interactions. Mated females with levels of ovarian development consistent with active queens were not involved in aggression, either as actors or as recipients, a finding in accord with that of Herman et al. (2000). Rather, the mated females involved in aggressive interactions had poorly developed ovaries. The number of queens on *P. colobopterus* nests cycles temporally, with the production of new queens occurring only when there are few or no queens remaining (West-Eberhard 1978; Strassmann et al. 1991, 2002; Queller et al. 1993). At this time newly emerged females
mate as a distinct age cohort. The poorly developed ovaries of the mated aggressors and victims indicate that some females from this cohort do not actually become reproductively active queens at all, or are quickly forced out of that role. Our findings along with those of Herman et al. (2000) and Strassmann et al. (2002) suggest that aggression is the mechanism suppressing these mated females. Since workers are unable to discriminate matrilines within the colony (Strassmann et al. 1997), it may be that workers suppress these mated females from being active queens because they are less fertile and thus are less likely to be their mother (Forsyth 1978; Noll & Zucchi 2000). The role of mated females as aggressors suggests that these females may use aggression to suppress one another in competition over reproductive rights (West-Eberhard 1978, 1981). Alternatively, once mated females have been repressed from being an active queen, they themselves may become active in suppressing other females from developing their ovaries.

Conflict involving unmated females

Females with ovarian development suffered a disproportionately large share of aggression, particularly when they were young. If these unmated females were not repressed, they could produce male eggs, or they could mate and produce females. It is generally in the other workers’ interests to prevent young females from producing either males or females as long as there are viable queens in the colony (Strassmann et al. 2002). This study and the finding that the experimental removal of all queens leads to decreased aggression and more ovarian development and mating in young females suggests that aggression is linked to the conflict over who becomes a queen (Strassmann
et al. 2002). Females involved in aggressive interactions (both victims and aggressors) have more ovarian development than their randomly sampled female colonymates, but not nearly as much as queens have. Victims are younger and aggressors are older than their randomly-sampled female colonymates. Aggressive interactions involve older females attacking younger females, with both parties usually having some ovarian development.

We did not observe a relationship between relatedness and the per capita frequency of aggression involving females, indicating that there may be constant suppression of newly emerging workers at many stages of the queen number cycle. However, we may not have detected such a relationship between aggression and relatedness because only one of our 34 colonies had very high relatedness, indicating low queen number, during the study period. We did find, however, that a higher proportion of young females have ovarian development when average colony relatedness is higher and thus queen number is lower, a result also reported in Strassmann et al. (2002). This increase in proportion of females with ovarian development could be related to the imminent production of queens.

In *P. colobopterus* there is a substantial payoff motivating emerging females to become queens, but allowing this is in the genetic interests of existing workers only when queen number is low, or zero, based on both the benefits of split sex ratios (Queller et al. 1993) and the relatedness ratios associated with replacing old queens with new queens (Strassmann et al. 2002). Thus, without a mechanism regulating queen production a tragedy of the commons of too many queens could occur, resulting in decreased colony function (Frank 1995; Bourke and Ratnieks 1999; Ratnieks 2001; Strassmann et al. 2002;
Wenseleers, unpublished data). The aggressive suppression of emerging females could serve as a mechanism by which older workers prevent others from becoming queens, thereby preventing untimely production of queens (West-Eberhard 1978; Herman et al. 2000; this study). Aggression decreases following the experimental removal of queens from the colony, indicating that when there are no queens on the nest workers reduce suppression of ovarian development in emerging females, thereby allowing them to become queens (Strassmann et al. 2002). This process results in the cyclical oligogyny pattern that typifies these wasps (West-Eberhard 1978; Queller et al. 1993).

*Other studies of aggression in social insects*

Though our data supports the hypothesis that genetic conflicts of interest motivate aggression, this is not necessarily the only motive for aggression. Several studies of other epiponine wasps indicate that aggressive interactions have a role in stimulating and organizing polyethism (Jeanne 1991; O'Donnell, 2001a). In *Polybia occidentalis* biting interactions on the exterior nest surface have been shown to stimulate victims to begin foraging and is not related to the ovarian development of either the aggressor or the victim (O'Donnell & Jeanne 1995; O'Donnell, 2001a). This finding does not, however, exclude the possibility that young, developing females are the targets of aggression inside the nest envelope (O’Donnell 2001b), as we found in this study of aggressive interactions occurring inside the colony. Young females may be attacked within the nest causing ovarian regression and driving them to the nest exterior where further aggression stimulates them to forage.
Ovarian development appears to be a general trigger for aggression as workers police other workers attempting to lay eggs. Studies in ants, bees, and wasps have indicated that workers with ovarian development are more often the target of attacks from other workers than are workers without ovarian development (bees: Velthuis 1976; Visscher & Dukas 1995; ants: Cole 1981; Franks & Scovell 1983; Crosland 1990; Gobin et al. 1999; wasps: Pardi 1948; Barth et al. 1975). Moreover, other studies have demonstrated cases where larvae developing into gynes receive aggression from workers in polygynous fire ants and Argentine ants (Fletcher 1986; Vargo & Passera 1991). Such attacks suggest a form of policing that suppresses females from developing their ovaries and reproducing.

It is interesting that control of worker reproduction is so often behavioural, and not entirely fixed during development. This may indicate that emerging females will be allowed to reproduce often enough that complete ovarian suppression should not be fixed. If queens die, or become unproductive, the colony can salvage some reproduction only by having plastic workers able to reproduce. Reproductive plasticity seems to generally be limited to young workers, as was the case in our study, though this is not always the case. For example, in Polistes exclamans older workers have more ovarian development and take over if the queen dies (Strassmann & Meyer 1983).

Worker collective control

This study suggests that workers use aggression to regulate the timing of queen production in accord with their interests. Several other forms of reproductive conflict in *P. colobopterus* have previously been investigated. Worker collective interests are also
satisfied in conflict over the timing of male production (Queller et al. 1993) and conflict over who produces males (Henshaw et al. 2000). Such decentralized colony control has likely been important in the evolution of social insect societies by facilitating colony level adaptations and thereby the emergence of complex societies (Jeanne, in press).
CHAPTER 2

Environmental heterogeneity and the coexistence of cheaters and victims in the social amoeba *Dictyostelium discoideum*

ABSTRACT

Conflict among cells is not expected in most multicellular organisms because they develop clonally from a single cell. The cellular slime mold *Dictyostelium discoideum*, however, develops by aggregation and can form a chimeric multicellular organism in which different clonal lineages compete for reproduction. In chimeric mixtures of two clones, one clone is often over-represented in the fertile spore cells and previous work has shown that clones can be ranked in a linear dominance hierarchy according to their ability to exploit other clones. This raises the question of how clones at the bottom of the competition hierarchy, which are consistently cheated of reproduction by others, can persist. Here we examine the possible role of environmental heterogeneity in the coexistence of cheaters and victim clones by examining the outcome of 8 pairwise competitions in 8 different environmental contexts. In some cases, exploitation by one clone is robust to environmental conditions. However, we also find evidence that the reproductively dominant clone in a chimera can change depending on the environment in which they aggregated. This helps to explain the coexistence of cheaters and victims in nature and suggests that selective pressures for exploitation vary across the landscape.
INTRODUCTION

Cooperative alliances have been central to many key events in the history of life. On several occasions, independent units have coalesced giving rise to evolutionarily successful higher levels of biological organization (Buss 1987; Maynard Smith & Szathmáry 1995). Prokaryotes have come together to form eukaryotes, single cells have joined to form multicellular individuals, and individuals have formed societies (Maynard Smith & Szathmáry 1995). However, unless cooperators are genetically identical, conflict is predicted because each party’s interests favor exploiting the relationship (Keller et al. 1999). Because of this tension, the stability of cooperative alliances requires control of such cheating (Maynard Smith & Szathmáry 1995; Michod & Roze 1997).

The single-cell bottleneck found in most multicellular organisms removes the potential threats of conflict and cheating since, barring high mutation rates, all the resulting cells are genetically identical (Michod 1996, 1997). However, chimeric multicellular organisms do occur and in such organisms there is often reproductive conflict among cells from different clonal lineages (Stoner & Weissman 1996; Grosberg & Strathmann 1998; Stoner et al. 1999; Velicer et al. 2000; Strassmann et al. 2000). The social amoeba, *Dictyostelium discoideum*, readily forms chimeras. Typically *D. discoideum* exists as free-living amoebae that prey upon bacteria in forest soils and leaf litter. However, when starved, thousands of cells aggregate to form a migrating slug that morphs into a fruiting body, composed of a stalk of dead cells that support a mass of reproductive spores.

In nature, clonal diversity is high and strains co-occur at small spatial scales, suggesting that genetically distinct clones often co-aggregate (Fortunato et al. in press a).
Furthermore, laboratory studies have shown that genetically distinct strains of *D. discoideum* will always aggregate to form chimeric fruiting bodies (Strassmann et al. 2000). Strains that attain unfair proportions of reproduction in controlled laboratory conditions are relatively common (Strassmann et al. 2000; K. Foster, T. Platt, J. Strassmann, & D. Queller in preparation). Moreover, if clones of *D. discoideum* are competed against each other in all combinations (round-robin) a linear dominance hierarchy is found (Fortunato et al. in press b; K. Foster, T. Platt, J. Strassmann, & D. Queller in preparation). This suggests that some clones consistently cheat others of reproduction in chimeras and raises the question of how victims and cheaters can co-exist in nature. That is, why don’t victim clones go extinct?

Several factors may promote the coexistence of victims and cheaters. One possibility is that cheaters suffer costs at another life history stage. However, measurements of vegetative and developmental characters of cheats and victims have failed to find evidence for any such pleiotropic effects of cheating genotypes (K. Foster, T. Platt, J. Strassmann, D. Queller in preparation).

Alternatively the selective pressures acting against victims may be weak. One reason that this might be true is if chimerism is rare in nature. This seems unlikely, however, since natural populations of *D. discoideum* harbor high clonal diversity at small spatial scales and in the laboratory clones always readily co-aggregate (Strassmann et al. 2000; Fortunato et al. in press a, b). Another possible reason that selective pressures against victim strains may be weak is if cheaters in one context become victims in another. Wild strains of *D. discoideum* are exposed to substantial spatial and temporal environmental heterogeneity (Raper 1984). Cheaters might not be able to exploit in all
chimeras if the environment in which strains co-aggregate influences the outcome of competition.

In this study we examine the outcome of competition between pairs of *D. discoideum* clones in different environmental conditions. We set up equal mixtures of 8 different pairs and then assayed the outcome of competition of these mixes under different pH, moisture, and temperature. Three of eight pairs of strains had a robust outcome to competition across all environmental conditions. However, in the five others, the outcome of competition changes depending on the extrinsic factors. This suggests that environmental heterogeneity helps maintain cheater and victim phenotypes in a population of cooperating individuals.

**METHODS**

**Summary**

To evaluate the effect of different extrinsic factors on the outcome of competition between co-aggregating strains of *D. discoideum*, we competed 8 different pairwise mixes of clones under varying moisture, temperature, and pH conditions. Equal numbers of each strain were mixed in pairs and each pair was placed on a nutrient-free plate where they starved, aggregated, and developed into fruiting bodies. Using a microsatellite marker, we estimated the representation of the two clones before and after aggregation, which allows us to assess whether one of the clones increased its representation in the spores relative to the initial mixture of cells.
Obtaining amoebae of *D. discoideum*

We used 9 genetically different strains, isolated from forest soils near Mountain Lake Biological Station (37°22'32''N, 80°31'20''W) in southwestern Virginia in the summer of 2001 (Fortunato et al. in press a). Immediately following isolation from nature, we stored these strains as frozen spores at −80°C. To obtain fresh, unfrozen spores to grow vegetative cells for experimental trials, we grew each strain from its frozen spore stock using 200μL of fresh overnight culture of the bacterium *Klebsiella aerogenes* as prey on 100x15 mm Petri dishes prepared with autoclaved SM/5 medium (Glucose 2g, Oxoid Bacto peptone 2g, Yeast extract 2g, MgSO₄·7H₂O 0.2g, KH₂PO₄ 1.9g, K₂HPO₄, Bacto agar 20g, H₂O to 1000mL, pH 6.4) at room temperature (approximately 22°C). To do this we simply inoculated spores into the 200μL of fresh overnight culture and evenly spread this suspension over the plate’s surface. We allowed the resulting amoebae to starve and then undergo complete development to fruiting bodies.

To obtain amoebae for experimental trials, we used the fresh spores from 5 of these sori to culture cells on 100x15 mm SM/5 plates, again using 200μL of *K. aerogenes* fresh overnight as prey. We first used sterile pins to transfer these 5 sori to a tube containing 150μL of deionized water. We allowed the spores to sit in the water for approximately 30 minutes while we prepared the Petri plates with bacteria. We aliquoted the 200μL of *K. aerogenes* fresh overnight onto the SM/5 plates and used a flame sterilized glass bar to spread this bacteria suspension evenly over the plate. After the bacteria suspension had dried we then transferred and evenly spread the spore suspension over the surface of the plate.
Twenty-eight hours after placing the spores on the nutrient plates with *K. aerogenes* we washed the agar medium surface with autoclaved KK2 buffer (2.25g KH$_2$PO$_4$, 0.67g K$_2$HPO$_4$ to 1000mL, pH 6.1) to collect the amoebae in the vegetative phase, prior to beginning differentiation. We then filled each tube with additional KK2 buffer to 20mL. To remove the bacteria we centrifuged each suspension (311 x g for 5 minutes), poured off the supernatant, and resuspended the pellet in 20mL of KK2 buffer. We repeated this wash twice wash, but following the final wash we instead resuspended the pellet in 1mL of KK2 buffer. We then used a hemocytometer to estimate the density of amoeba for each suspension. For each estimation we made counts from 2 separate aliquots of the cell suspension. To avoid biasing counts, for each aliquot we always counted the center square and the 4 corner squares.

*Mixing experiments*

We mixed 8 pairs of clones in equal numbers by placing an 100μL aliquot (containing 3 million cells of each strain) of each mixture on a 60 x 15 mm Petri plate prepared with autoclaved starving medium (0.356g Na$_2$HPO$_4$, 1.98 KH$_2$PO$_4$, 20 g agar, in 1000mL H$_2$O). We competed each pair of clones in 8 different environmental conditions (Table 4) at a density of approximately 2 million cells/ cm$^2$. We did not spread the cells across the plate but rather simply delivered the aliquot onto the center of the agar surface of the Petri plate. We allowed strains to co-aggregate and form fruiting bodies at three different temperatures (10°C, 15°C, and approximately 22°C), three different acidities (pH 4.5, 6.0, and 6.4), and four moisture levels (Table 4). As a control that all strains
were able to develop in each of the environmental conditions, we also plated out 6
million cells of each strain clonally in each of the 8 environmental conditions.

Table 4. Description of different environmental conditions

<table>
<thead>
<tr>
<th>Environment</th>
<th>Vol. water added (μL)</th>
<th>pH</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab norm</td>
<td>0</td>
<td>6.4</td>
<td>≈ 22</td>
</tr>
<tr>
<td>pH 4.5</td>
<td>0</td>
<td>4.5</td>
<td>≈ 22</td>
</tr>
<tr>
<td>pH 6.0</td>
<td>0</td>
<td>6.0</td>
<td>≈ 22</td>
</tr>
<tr>
<td>125 μL</td>
<td>≈125</td>
<td>6.4</td>
<td>≈ 22</td>
</tr>
<tr>
<td>375 μL</td>
<td>≈375</td>
<td>6.4</td>
<td>≈ 22</td>
</tr>
<tr>
<td>625 μL</td>
<td>≈625</td>
<td>6.4</td>
<td>≈ 22</td>
</tr>
<tr>
<td>10°C</td>
<td>0</td>
<td>6.4</td>
<td>10</td>
</tr>
<tr>
<td>15°C</td>
<td>0</td>
<td>6.4</td>
<td>15</td>
</tr>
</tbody>
</table>

To establish the four moisture treatments we used an atomizer to spray either
approximately 0, 125μL, 375μL, or 625μL of water evenly across the agar surface of the
Petri plate. At low pH and high temperatures agar polymerization is unstable. Thus, in
order to attain stable pH 4.5 and pH 6.0 agar plates we used high quality plant
propagation agar (Marine Bioproducts, P-01-500) and autoclaved the acidic starving
medium and the agar solution separately, combining them only after both had cooled. For
all other environment treatments we used Bacto Agar and autoclaved the agar with the
starving medium.

Genetic analysis

We gauge cheating by comparing the proportion of each strain in the initial cell
mixture to that of the resulting spore population. To estimate these proportions we first
extracted DNA from both 50μL of the initial cell suspension of equal numbers of each
strain. To do this we first centrifuged the cell suspension at 10,600 x g for 5 minutes to
remove salts. We then resuspended the pellet in 150μL of 5% Bio-Rad Chelex and 5μL of 20mg/mL Proteinase K and heated this at 56°C for 4 hours and subsequently 98°C for 30 minutes. To estimate the proportion of each strain in the spore population, after development we extracted DNA from 8 sets of 5-7 combined sori of the resulting chimeric fruiting bodies for each of the 8 environment treatments. To do this we used vigorous vortexing to disperse the sori in 150μL of 5% Bio-Rad Chelex and 5μL of 20mg/mL Proteinase K and heated as before. We then amplified a microsatellite locus for which the two strains had different alleles using fluorescently labeled primers (Dicty25.AAC) in a polymerase chain reaction (PCR) (44 cycles: 95C for 30s, 49C for 30s, 72C for 30s).

We quantified the relative proportions of each allele in both the initial cell suspension and the resulting spore population using an ABI Prism® 3100 Genetic Analyser. PCR preferentially amplifies shorter products over longer ones. Consequently the area ratio of alleles present after PCR may not correspond directly to the proportion of each clone present. To correct for differential amplification we adjusted the ratio of the 2 alleles’ area from each spore population estimate according to the allelic proportions from the initial cell suspension, which was a fifty-fifty mixture of the two strains. To do this we calculated a weighting factor that adjusted the cell suspension’s allele area ratio to 0.5 and then multiplied this weighting factor by each spore population allele area ratio.

For a given competition we classified a strain that composes significantly more than 50% of the spore population as a cheater and any strain that composes significantly less than 50% as a victim.
Statistical methods

All statistics done on proportions were first arcsine square root transformed to normalize the data (Sokal & Rohlf 1981). When examining the significance of contrasts between the outcomes of competition in two different environmental conditions, we used the Bonferroni-Holm sequential method to correct for multiple comparisons (Holm, 1979; Sokal & Rohlf 1981).

RESULTS

Averaging across all environments, in 5 of the 8 competitions one clone was able to get statistically more representation in the spore population than was fair (t-test, p<0.05 for V55C2 vs. V326D1, p<0.0001 for V324B1 vs. V55C2, V324B1 vs. V326D1, V327A1 vs. V336B1, V336B1 vs. V77B; Figure 3).

Despite this, environmental conditions altered the outcome of several competitions (Table 5; Figures 4-8). In 2 of the 8 cases, one strain behaved as both a cheater and a victim depending on the environment in which the strains interacted (Figures 4-5). In 3 other cases, only one of the clones ever cheated but the degree of exploitation changed across environments and in some environments there was no significant cheating effect (Figures 6-8). In another competition one strain was able to significantly exploit in 7 of 8 environments (Figure 9). Thus these experiments demonstrate that one strain can exploit across a wide range of environmental conditions (Figures 6-9), even if the environmental conditions in which two strains co-aggregate can significantly affect the outcome of the interaction and potentially change which clone exploits the other (Table 5; Figures 4-5).
Table 5. Analysis of variance for environment effects on the change in each strain’s proportions between the initial cell suspension and the spore population for each pair of competing clones. All sources of variance are nested by the pair of competing clones.

<table>
<thead>
<tr>
<th>Pair</th>
<th>DF</th>
<th>Mean Square</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>V55C2 vs. V326D1</td>
<td>7</td>
<td>0.208</td>
<td>7.488</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V342B2 vs. V301B2</td>
<td>7</td>
<td>0.271</td>
<td>5.330</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>V324B1 vs. V55C2</td>
<td>7</td>
<td>0.116</td>
<td>7.282</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>V324B1 vs. V326D1</td>
<td>7</td>
<td>0.268</td>
<td>3.546</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>V327A1 vs. V336B1</td>
<td>7</td>
<td>0.113</td>
<td>2.403</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>V336B1 vs. V77B</td>
<td>7</td>
<td>0.003</td>
<td>1.418</td>
<td>0.2286</td>
</tr>
<tr>
<td>V301B2 vs. V56A2</td>
<td>7</td>
<td>0.004</td>
<td>2.078</td>
<td>0.0679</td>
</tr>
<tr>
<td>V327A1 vs. V77B</td>
<td>7</td>
<td>0.002</td>
<td>1.365</td>
<td>0.2536</td>
</tr>
</tbody>
</table>
Figure 3. Average change, across all environments, in the proportion of the first clone between the initial cell suspension and the spore population for each of the competitions. Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.0001). Error bars are standard errors.
Figure 4. Average change in the proportion of strain V55C2 when in competition with strain V326D1 in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. Lack of a common letter indicates that the outcomes of competition in the two environments are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, all p<0.01; except p<0.05 for pH 6.0 versus 3 Sprays). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.01, and *** denotes p<0.001). Error bars are standard errors.
Figure 5. Average change in the proportion of strain V342B2 when in competition with strain V301B2 in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. Lack of a common letter indicates that the outcomes of competition in the two environments are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, p<0.01 for pH 6.0 versus 5 Sprays and pH 6.0 versus 15°C; p<0.05 for pH 6.0 versus Lab norm and 5 Sprays versus 10°C). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.001). Error bars are standard errors.
Figure 6. Average change in the proportion of strain V324B1 when in competition with strain V55C2 in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. Lack of a common letter indicates that the outcomes of competition in the two environments are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, all p<0.01; except p<0.05 for 1 Spray versus 5 Sprays and pH 4.5 versus Lab norm). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.01, and *** denotes p<0.0001). Error bars are standard errors.
Figure 7. Average change in the proportion of strain V324B1 when in competition with strain V326D1 in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. Lack of a common letter indicates that the outcomes of competition in the two environments are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, p<0.01 for 1 Spray versus 15°C and 10°C versus 15°C; p<0.05 for Lab norm versus 15°C). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.01, ** denotes p<0.0001). A circled asterisk indicates that the entire spore population was composed of only one strain, V326D1, indicating strong exploitation but making statistical analysis impossible due to lack of variance. Error bars are standard errors.
Both competitions involving strain V336B1 suggest that this clone is able to exploit under a wide range of environmental conditions (Figures 8-9). Strain V336B1 significantly exploited strain V77B in 7 of the 8 environments assayed (Figure 9) and strain V327A1 in 5 of the 8 environments (Figure 8), though in the latter competition there was a significant effect of environment (Table 5).

Figure 8. Average change in the proportion of strain V327A1 when in competition with strain V336B1 in 8 different environmental contexts. Environmental conditions had a significant effect on the outcome of exploitation (ANOVA, F= 2.403, p<0.05; Table 5). However, the outcomes of competition in none of the environments are significantly different from one another (Fisher's PLSD with a Bonferroni-Holm correction for multiple comparisons, all p>0.05). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.01, and *** denotes p<0.0001). Error bars are standard errors.
Figure 9. Average change in the proportion of strain V336B1 when in competition with strain V77B in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. The outcomes of competition in none of the environments are significantly different from one another (Fisher's PLSD with a Bonferroni-Holm correction for multiple comparisons, all p>0.05). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.01, and *** denotes p<0.0001). Error bars are standard errors.
In two competitions, the outcome of competition was close to fair in all environments (Figures 10-11). In these mixes the effect sizes of average changes in strain proportions were always slight, never exceeding 0.056 (Figures 10-11).

Figure 10. Average change in the proportion of strain V301B2 when in competition with strain V56A2 in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. The outcomes of competition in none of the environments are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, all p>0.05). Asterisks indicate a change in strain proportion significantly different from 0, indicating exploitation (t-test, * denotes p<0.05, ** denotes p<0.01). Error bars are standard errors.
Figure 11. Average change in the proportion of strain V327A1 when in competition with strain V77B in 8 different environmental contexts. ANOVA examining effect of environment on the outcome of competition reported in Table 5. The outcomes of competition in none of the environments are significantly different from one another (Fisher's PLSD with a Bonferroni-Holm correction for multiple comparisons, all p>0.05). An asterisk indicates a change in strain proportion significantly different from 0, indicating exploitation (t-test, p<0.05). Error bars are standard errors.

The degree of cheating (absolute change in clone proportions) varied across environments (Table 6). The least cheating occurred in the pH 4.5 treatment and this was significantly less than the level of cheating in the 1 Spray (Fisher’s PLSD with Bonferoni-Holm correction, p<0.01), 3 Sprays (Fisher’s PLSD with Bonferoni-Holm correction, p<0.01), 10°C (Fisher’s PLSD with Bonferoni-Holm correction, p<0.01), and 15°C (Fisher’s PLSD with Bonferoni-Holm correction, p<0.01) treatments (Figure 12). Similarly across all mixes, the average magnitude of clonal proportion change was lower for the pH 6.0 treatment than for the 3 Spray (Fisher’s PLSD with Bonferoni-Holm correction, p<0.05; Figure 10).
Figure 12. Average magnitude, across all 8 mixes, of changes in clone proportions during development for each of the 8 environments. ANOVA examining effect of environment on the outcome of competition reported in Table 6. Lack of a common letter indicates that two bars are significantly different from one another (Fisher’s PLSD with a Bonferroni-Holm correction for multiple comparisons, all p<0.01; except p<0.05 for pH 6.0 versus 3 Sprays). The average magnitude of change in strain proportions for all environments is significantly different from 0, indicating exploitation (t-test, p<0.0001). Error bars are standard errors.

Table 6. Influence of environment and the identity of the clone pair on the absolute magnitude of changes in clone proportions. The absolute magnitude of changes in clone proportions is calculated as the absolute value of the difference of one strain’s proportion between the initial cell suspension and each replicate estimate of the final spore population.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment</td>
<td>7</td>
<td>0.040</td>
<td>3.347</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pair of clones</td>
<td>7</td>
<td>0.856</td>
<td>72.466</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Environment*</td>
<td>49</td>
<td>0.035</td>
<td>2.950</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pair of clones</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
All strains plated clonally developed in all environmental conditions assayed. Thus changes in the proportion of a strain in the initial cell suspension and the final spore population were not simply the result of one strain failing to undergo development.

DISCUSSION

Wild strains of *D. discoideum* form a linear dominance hierarchy according to their ability to exploit under controlled laboratory conditions (Fortunato et al. in press b). This finding suggests strong selective pressures against victims of exploitation. Considering this, how do victims and cheaters coexist in natural *D. discoideum* populations? This study shows that environmental conditions can affect the outcome of conflict between co-aggregating genetically distinct clones of *D. discoideum*. Because the outcome of competition varies across different environments, victims are not exploited equally strongly in all chimeric aggregations and may even be a cheater in some environments. Thus the effect of environment on exploitation indicates that selective pressures acting against victims vary in the wild. Selective pressures are likely to vary spatially across the variety of substrates (e.g. soil, leaf litter, and scat) that *D. discoideum* live on (Raper 1984). Similarly selective pressures likely vary temporally with the seasonal and stochastic variation in moisture and temperature that they experience in the wild. Thus environmental heterogeneity may promote the coexistence of cheaters and victims by weakening the force of selection against victims, making it less likely that they go extinct.

Several other factors likely contribute to the coexistence of victims and cheaters in nature. A cell’s nutritional state (Blaschke et al. 1986; Inouye & Takeuchi 1982; Leach
et al. 1973) and cell cycle (Gomer & Firtel 1987; Maeda et al. 1989; Zimmerman & Weijer 1993; Araki et al. 1997) are known to influence whether it becomes a spore or stalk cell. In general, well-nourished cells that are preparing to divide (e.g. those in late G2 of the cell cycle) tend to become spores (Kessin 2001). Our experiments control for these cell history factors of cell-fate since we cultured all strains identically. However, a cell’s history is likely to be important in determining the outcome of competition in D. discoideum. For example, if an exploitative clone is more starved than the clone it is co-aggregating with then it probably will not be able to cheat. Thus, like environmental heterogeneity, stochastic variation in cell-history would also promote the coexistence of cheaters and victims by weakening the selective pressures against victims. However the degree to which it does so may be slight since co-aggregating strains are in the same microenvironment. Yet, if different environments differentially affect the nutritional or cell cycle status of D. discoideum strains, then this could be one reason that the environment affects the outcome of competition between strains.

Similarly, if different strains are spatially scattered and consequently chimerism occurs only infrequently in the wild, it may be that the selective pressure acting against victims is weak. This too would promote clonal diversity as it sets up a non-equilibrium state in which victim clones continue to persist in the population. This possibility seems unlikely, however, since genetically distinct clones have been found very close to one another in nature and in the laboratory clones always readily co-aggregate when starved (Strassmann et al. 2000; Fortunato et al. in press a, b). A cost to cheating at some other life history stage would also promote clonal diversity. Yet we have examined many vegetative and developmental characteristics of clones that cheat under controlled lab
conditions and failed to document any such cost (K. Foster, T. Platt, J. Strassmann, & D. Queller in preparation). Such costs, therefore, may not occur. However, given that a cheat genotype can only be identified through examination of their phenotype in a range of environments it is hard exclude this possibility.

If cheating is environmentally dependent, do true cheaters exist in wild *D. discoideum* populations? In several of our competitions the same clone significantly exploited in multiple environmental conditions, while the other clone failed to cheat in any environment (Figures 6-9). Even if these clones do not cheat under all environmental conditions, on average they are clearly doing better than the strain that they are exploiting (Figure 3). Thus our data indicate that some clones may have a consistent exploitative advantage in natural multicellular aggregations. Other interesting questions for future research is how frequently such robust cheating occurs and whether such cheaters are able to exploit many *D. discoideum* strains. Competitions involving strain V336B1 show that this is possible, as this strain is able to exploit under a wide range of environmental conditions and against both genotypes we paired it with (Figures 8-9) but from current data we cannot conclude whether such cheating is rare or common.

Our experiments suggest that environmental heterogeneity helps to account for the coexistence of cheaters and victims found in natural *D. discoideum* populations. Despite this, some clones do seem to be able to, on average, exploit more than other clones. Selective pressures against victims may be weakened by environmental heterogeneity and variation in cell-history, allowing drift to dominate this selective disadvantage and victims to persist in the population. Our experiments enabled us to show unequivocally that cheating is sometimes environmentally dependent. However, the
environment did not always affect the outcome of competition and an interesting question for future research is to evaluate the full extent of the environment’s influence on competition and whether persistent cheating occurs.
REFERENCES


Araki, T., Abe, T., Williams, J.G., & Maeda, Y. 1997. Symmetry breaking in
_Dictyostelium_ morphogenesis: evidence that a combination of cell cycle stage and

promotes dominance behavior and ovarian development in social wasps (_Polistes
annularis_). Experientia, 31, 691-692.

proportioning, cell-differentiation preference, cell fate, and the behavior of

University Press.


Oxford: Blackwell.

Bourke, A.F.G. 1999. Colony size, social complexity and reproductive conflict in social
insects. Journal of Evolutionary Biology, 12, 245-257.


differentiation of mixtures of metabolically distinct populations of *Dictyostelium

*Dictyostelium* cells to differentiation phase at a particular position of the cell
cycle. Differentiation. 41: 169-175.

Freeman.


Michod, R.E. 1996. Cooperation and conflict in the evolution of individuality. II. Conflict

Michod, R.E. 1997. Cooperation and conflict in the evolution of individuality. I. Multi-

Berlin: Springer-Verlag.

progression in some neotropical swarm-founding polygynic polistine wasps
(Hymenoptera, Vespidae, Eppiponini. Ethology, Ecology & Evolution, 12(1), 43-
65.

O’Donnell, S. 1998. Reproductive caste determination in eusocial wasps (Hymenoptera:
O'Donnell, S. 1999. The function of mail dominance in the eusocial wasp,  


