The Multi-level Selection debate illustrated by the different degrees of eusociality in the wasp family Vespidae.

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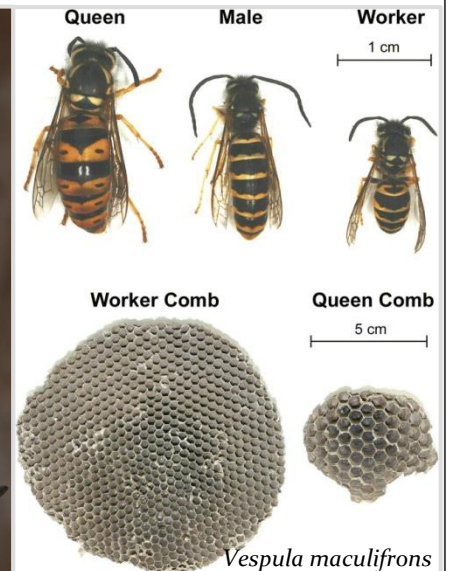*Euodynerus foraminatus*

Eustenogaster calyptodoma

Parischnogaster sp.

Queen   Male   Worker

1 cm

Worker Comb   Queen Comb

5 cm

Vespula maculifrons

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Abstract

In the evolution of eusociality, a couple of mechanisms are thought to play an important role. Here, I will focus on how two types of selection processes (MLS 1 vs. MLS 2) might work within species showing different degrees of eusociality.
Both selection processes point towards the importance of group-level processes, but where MLS 1 stresses that due to population structuring into groups the frequency of an individual trait might change differently than without structuring, MLS2 sees the group itself as a sole unit of selection. For four wasp species of the family Vespidae- all showing a different level of sociality in their colonies- I investigated how the described population fits into the MLS-framework. During this investigation, I took into account the underlying assumptions of the different Multi Level Selection processes. I conclude that complex Multi-Level Selection situations arise in all of the four cases, but none of them meets the strict criteria of the concept of MLS 2.

Introduction
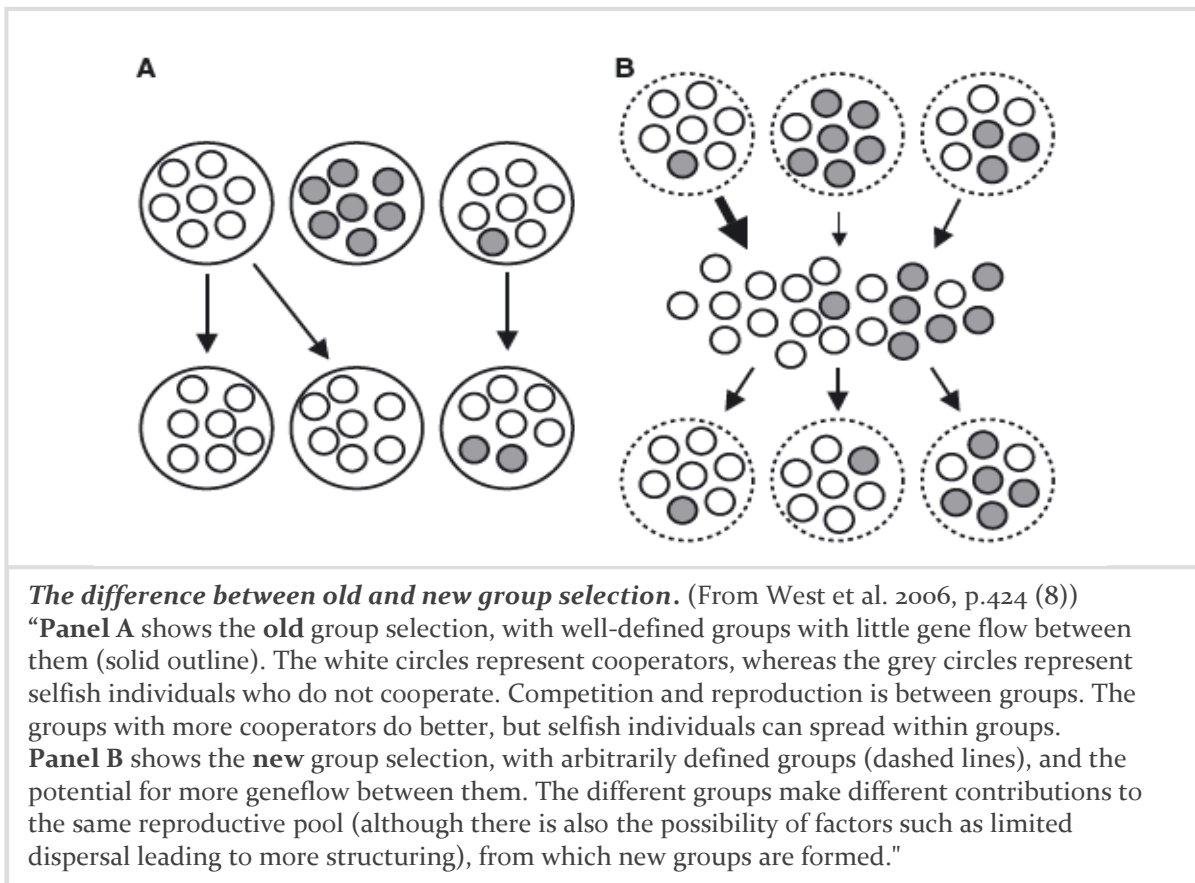
Selection at different levels.

When we follow the changes of a certain trait within a population over time, we could try to distill the more important factors *causing* the change from an enormous bulk of potential mechanisms (e.g. migration, drift, environmental changes, selection pressures, etc). In this thesis, I will focus on two different types of selection processes.
Natural Selection, as the process proposed by mister Darwin in the 19th century (1), is nowadays most commonly understood as the process in which individuals within a certain population have different viabilities and reproductive output due to their different –heritable- phenotypes. In other words, for the process of natural selection to act, three requirements are needed;

1.) Heritability; the expressed value of the investigated trait has to be heritable, at least to some extent. We normally assume that there is a genetic basis for the trait; the genes are thus the heritable information (see below for the criteria of 'units of selection'), which can be passed on to the next generation. 2.)*Variation;* in order for selection to act, there must be some (phenotypic) variation of the trait present in the population. 3.)*Differential survival/ reproduction* of the different variants of the trait. Natural selection requires an association between the investigated trait and its fitness. In other words; the variant having the greatest fitness in the population will be favored by natural selection. Its offspring needs to inherit not only the trait, but also the fitness-advantages associated with the trait. For direct selection, not only statistical correlation between the trait and its fitness is needed, but also causality; the trait itself needs to *cause* the higher fitness.

These Darwinian principles of variation, heritability and differential success can act on different hierarchical levels. As we saw, they might act on the individual level, but in theory, they could also hold for other levels being subject to selection.

In 1962 V.C. Wynne-Edwards proposed the idea of group selection (2): the process in which selection acts on the group level, in such a way that certain *groups* have a higher fitness than other *groups*. Since he proposed his ideas, there has been an intense debate about the question whether this process might truly work in nature. Critics as J. Maynard Smith (3) pointed out that both migration between groups and within group competition would works as processes eroding the between group variation necessary to have selection at the group level. In 1975, a new 'group-selection process' was proposed by D.S. Wilson (4). He argued that sub-populations that function better as a group (e.g. by containing more cooperative individuals) might deliver more individuals to the next generation.

The difference between old and new group selection. (From West et al. 2006, p.424 (8))
"**Panel A** shows the **old** group selection, with well-defined groups with little gene flow between them (solid outline). The white circles represent cooperators, whereas the grey circles represent selfish individuals who do not cooperate. Competition and reproduction is between groups. The groups with more cooperators do better, but selfish individuals can spread within groups.
Panel B shows the **new** group selection, with arbitrarily defined groups (dashed lines), and the potential for more geneflow between them. The different groups make different contributions to the same reproductive pool (although there is also the possibility of factors such as limited dispersal leading to more structuring), from which new groups are formed."

For clarity, I will use the terms as proposed by Damuth & Heisler (5) to clearly differentiate between the two processes; **MLS 1** (Multi-Level-Selection type **1**) to refer to Wilson's new group selection theory and **MLS 2** for the old group selection theory as proposed by Wynne-Edwards. For further explanation and discussion see (6), or (7), (8).

The group as an entity.
So far we have seen that there are different levels, or 'entities' on which we can focus in evolutionary analyses. What does it mean to call *groups* entities? First, the individuals within

the group need to be bounded together in a more than random way. Populations with low migration rates are a clear example; spatial separation can structure the individuals of a species into groups. Second, the group needs to be a reproductive unit, i.e. it needs to be able to reproduce (i.e. having baby-*groups*) and die. Obviously, the group has to last long enough to make these group-copies (9). And third, these groups need to exhibit clear 'group-traits' that are more than just the assembly of the individuals within the group. Off course, selection can act on these traits when the above described Darwinian principles are met; the group-trait needs to be heritable, there has to be a certain amount of variation of the trait between groups and it has to be associated with (or cause) the differential reproduction and survival of the groups.

Sociality and the wasp family *Vespidae*.

The problem of social behavior lies in the question why an individual should carry out a cooperative, or helping, behavior that appears costly to perform, but benefits other individuals (8) (10). This is the case in many social insect species, where daughters –or others- help their mothers at the nest. The degree at which this cooperative or social behavior is intergraded within the colonies of the insects differs among species (see Tables 1 & 2).

The most extreme form of cooperative behavior is called 'eusociality', meaning 'truly social' (Greek $\varepsilon \upsilon$ = true/good). Eusociality is commonly characterized by 1.) Individuals of the same species cooperate in caring for the young; 2.) there is a reproductive division of labor, with more or less sterile individuals working on behalf of fecund nestmates; 3.) and there is an overlap of at least two generations in life stages capable of contributing to colony labor, such that offspring assist parents during some period of their life (11) (12) (13). I have to point out however, that other definitions of eusociality exist as well, which try to solve the fact that "some complex forms of sociality did not precisely fit the three trait criteria" and focus more on "the trade-off between individual reproduction and cooperation" (14).

	Cooperative brood care	Castes	Generation overlap
Communal	No	No	No
Quasisocial	Yes	No	No*
Semisocial	Yes	Yes	No
Eusocial	Yes	Yes	Yes

Table 1. "The tradional categorization of social insects, from Michener and Wilson" (13). The different degrees of sociality are classified according to the most obvious characters

For this thesis, one species was chosen from each of three related subfamilies within the family *Vespidae* (*Eumeninae, Stenogastrinae, Vespinae* (15)), as an example for the different degrees of eusociality. "Wasps in the family *Vespidae* have played a central role in the understanding and development of the evolution of social habits. Of the more than 5,000 species in the family, social wasps comprise only a fifth; ... the majority of vespids are solitary." (15)

After a brief sketch of the population structure and the reproductive habits and cooperative tendencies of the species, I will clarify my statements about how the described population fit into the MLS-framework. During the investigation, I will take into account the different underlying assumptions of the different Multi Level Selection processes.

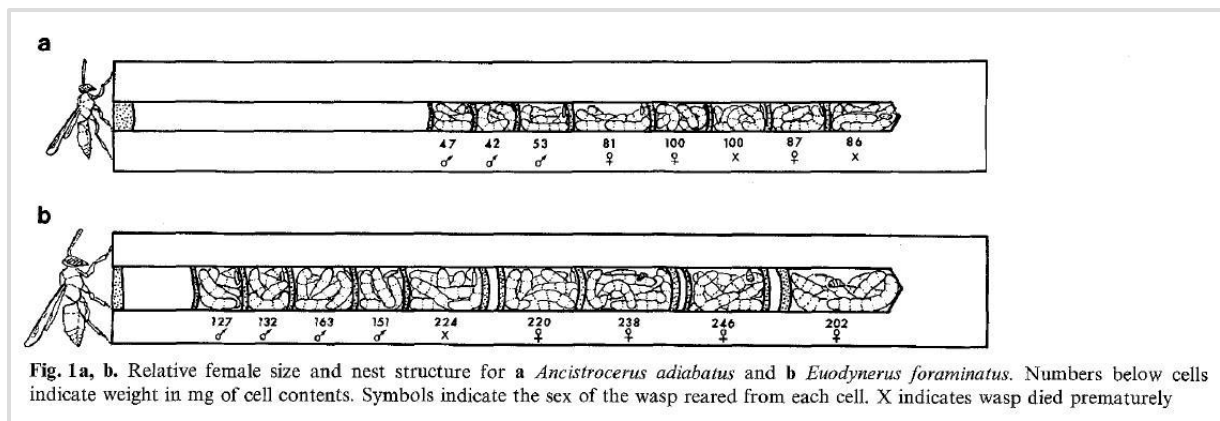|	Solitary *A. adiabatus &* *E. foraminatus*	Primitively eusocial *E. calyptodoma*	Facultatively eusocial *P. alternata*	Obligatory eusocial *V. maculifrons*
Nr. females per colony	1	1 (or 2!)	1-9 + 'queen'	3000 + queen
Cooperative brood care	-	+	+	+
Overlap of generations	-	+	+	+
Reproductive caste differentiation	-	Age based	Queue (age-based)	+
Queen-worker dimorphism	-	-	-	+
Sterile castes	-	-	-	±
Sex ratio	*Investment* ratio female biased.	Female biased	Female biased	Female biased
Inhibition of worker reproduction	-	-	"feasable"	Pheromonal
Mono- vs. Polyandrous	? uncertain: mate only once	Varying betw. populations		Polyandry; but see discussion.

Table 2. The presence of different –sociality indicating- traits within the studied populations of five wasp species in the family *Vespidae*, all showing different levels of social integration in their colonies.

Varying degrees of sociality & Multi Level Selection.

Solitary wasp; Ancistrocerus adiabatus & Euodynerus foraminatus.

"The solitary wasps *Ancistrocerus adiabatus* and *Euodynerus foraminatus*, both part of the sub-family *Eumenidae*, nests alone in small hollow twigs or vacant insect borings in wood. The nest within the hole consists of linearly arranged cells separated by walls of mud (Fig. 1 (16)). Inside each cell the female wasp lays one egg and places all the food for the growth and development of the larva to adulthood. When females complete one nest they move to another hole and start another series of cells. However, when all the trap nests in an area are occupied, females will enter and destroy the nests of other wasps, and use these holes to rear their own brood." (16)

"Males of *E. foraminatus*, upon emergence as adults, fight for control of the nest area. The winning brother remains at the nest and mates with his sisters as they emerge several days later. The available evidence indicates that females mate only once. Males of *A. adiabatus* are not as agonistic as males of *E. foraminatus* so that a number of males may remain near the natal nest. When females nest near each other, male-male interactions increase, and the likelihood of sibling mating decreases. Under conditions of clumped nesting, females make larger offspring and invest more in males than when nesting in isolation (see appendix for data)." (16)

Fig. 1a, b. Relative female size and nest structure for **a** *Ancistrocerus adiabatus* and **b** *Euodynerus foraminatus*. Numbers below cells indicate weight in mg of cell contents. Symbols indicate the sex of the wasp reared from each cell. X indicates wasp died prematurely

To investigate the evolutionary processes in these species, it is very obvious that one has to take into account the different 'choices' each *individual* can make; a female can choose a place to nest, lay a certain sexratio, invest in her brood, compete with other females for nest places... Males of *E. foraminatus* fight intense with brothers, but might even fight with males from adjacent nests. Although winning mainly depends on size, which is highly influenced maternal investment; time invested in defending a certain nest will certainly increase copulation chances and therefore its individual fitness.
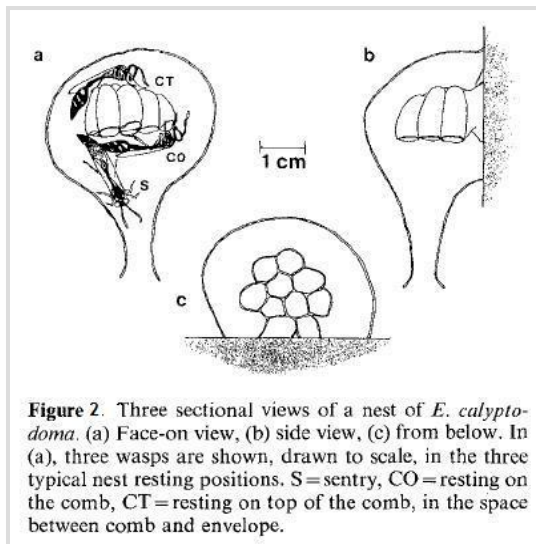
All these individual choices affect the fitness (i.e. its fecundity and survival) of a certain individual and might change in that way the distribution of certain traits within the population. As Wilson pointed out in his group-selection theory (MLS 1): by structuring a population into groups, the frequency of an individual trait might change differently over evolutionary time than without structuring. In other words; it is important to know in which "group situation" a certain individual choice is made. This holds true for this specific case: as we saw, females make larger offspring and invest more in males under conditions of clumped nesting than when nesting in isolation. The population density thus affects the reproductive choices of the individual and thereby the population structure of the next generation.

Primitively eusocial; Eustenogaster calyptodoma.

"In *Eustenogaster calyptodoma* (Stenogastrinae, Vespidae), nests are founded by a single female (fig. 2 (17) & cover picture (18)). Social groups arise from newly emerged females staying with the adult female already in residence; the oldest female guards the nest entrance while a younger female forages. All females have the potential to become sexually mature. Within a week of emergence, a young female generally leaves the nest to found her own colony, either by building her own nest or by usurping one *plus its brood* from another wasp." (17). - Note the difference with the solitary wasps, which destroyed the broods of usurped nests!
" Usurping brood appears to be a method of acquiring assistance quickly in the running of the colony, since emerging females might help the usurper foraging; a female building her own nest would have to wait more than 60 days for such assistance." (17)

The preference for usurping -and leaving the usurped brood intact- above self-founding seems reasonable, but; why would the helpers help? Newly emerged females will help the first days of their lives at the colony, but females who failed to found or usurp a nest themselves return to the maternal nest to help as well. The author suggest a few reasons why they start helping, *even if the nest is taken over by another female*:

Figure 2. Three sectional views of a nest of *E. calypto-doma*. (a) Face-on view, (b) side view, (c) from below. In (a), three wasps are shown, drawn to scale, in the three typical nest resting positions. S = sentry, CO = resting on the comb, CT = resting on top of the comb, in the space between comb and envelope.

1.) the younger female might just not recognize the usurper as non-kin,

2.) they have their own interest in the brood ("kinship effects") since the rest of the brood might be related to her, or

3.) females might learn by helping and therefore increase their own future reproductive success.

Although sample sizes were very low in this study, a significant female-biased sex ratio was observed in the hatching young wasps (2:1, females: males). Hansell suggest that this "could enhance the advantage to a female of staying in the maternal nest"; the relatedness to sisters will be ¾ and to brothers and own offspring ½. "Still, colonies of this wasp generally do not exceed two females- and that only temporarily. One possible reason for this may be the low degree of relatedness between colony members, due to polygyny and nest usurption." (17)

Thinking about group-level selection requires a description of what exactly we will call a 'group'. In the example of the solitary wasp, it was very obvious that individual wasps were organized into groups at the population level, with nest density as a 'group-trait'. In this situation, I can think of two different ways to define a 'group'.

First, since there seems to be a recognizable division of labor, in which the older female guards the entrance and the younger forages, one way of defining a 'group' could be to consider the founding (or usurping) female and her helper as a group. The raised progeny, which can consist of multiple unrelated layers due to usurption, could be in- or excluded from this definition. Central would be the view that these two females form a very small colony. But especially since cooperation is maintained for only a very short time, i.e. the group is showing no 'longevity', this 'group' can not be the basis of a MLS **2** process. Furthermore, since an important part of the reproduction-phase (male-male competition) takes place outside this 'group', this small group-definition also wouldn't be satisfying in a MLS **1** setting. In an MLS 1 setting, especially the reproductive-phase is centered within the group, whereafter the progeny can merge again in the bigger gene-pool.

As a second definition, a 'group' could be a sub-population, bounded together as group in space (e.g. defined by maximal dispersal distance). We can imagine a couple of nests together in a certain area, and define this sub-population as a group. There could be a small amount of migration to other areas, as in the MLS **1** setting described earlier (8). In *E. calyptodoma*, this could be a useful way of looking at the evolutionary dynamics; although no further explanation is given, the author states that the "success of usurpation is correlated with nest density" (17). When we consider nest density a sub-population (or 'group-') specific trait showing variation between groups, than it is not very hard to imagine that usurption and helping frequencies are different in different 'groups'. Assuming that these are heritable traits, we can imagine a MLS **1** process in which the group-state (e.g. nest density) influences the distribution of the individual traits (e.g. usurption, helping).

Facultatively eusocial; *Parischnogaster alternata.*

 "The colonies of the Malaysion hover wasps *Parischnogaster alternata* (Stenogastrinae, Vespidae) are small, consisting of 1–10 females. They may be long lived and occur often in dense clusters. Nests are founded by single females. When her eggs hatch, she proceeds to feed the larvae on captured prey. After pupation, newly hatched adults may disperse to found new nests or may become helpers on their natal nests, where they have the potential to become reproductives."

"Although all adult colony members are capable of mating and producing offspring, offspring-production was nearly always monopolized by a single dominant female. This high reproductive skew in *P. alternata* may result from
1.) strong ecological constraints on solitary nesting,
2.) high relatedness (subordinates gain indirect benefits when helping to raise the offspring of the dominant female), and
3.) a relatively high probability that subordinates will eventually inherit the position of the dominant egg-laying position." (19)

Parischnogaster sp. on nest (18)

The authors investigated the genetic of the population using microsatellite loci. They found that *P. alternata* subordinates were related to male eggs and small larvae by $r = 0.56 \pm 0.04$. (19)
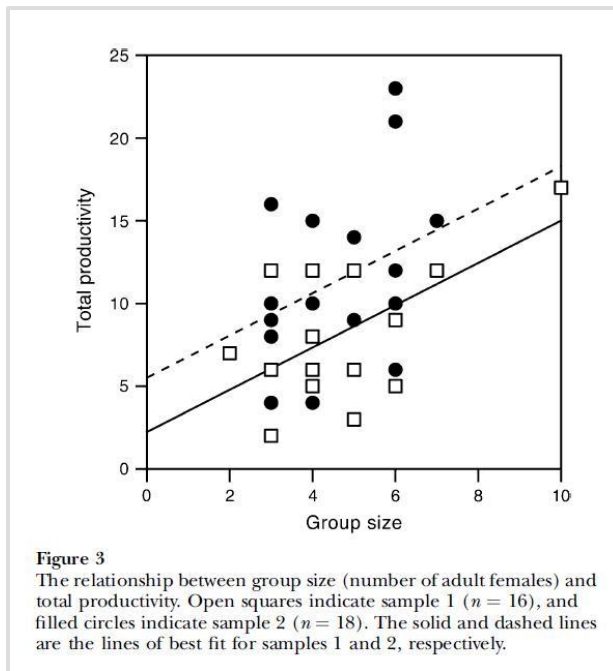Since *P. alternata* is a haplodiploïd species, each subordinate should have preferred her own sons ($r = 1.0$) to those actually being reared. The same would apply for the dominant female, preferring her own sons above others. Furthermore, since the cooperative group will often 'comprise mixtures of sisters, cousins, aunts, nieces, etc.' (subordinates are thus generally not the offspring of dominants), a similar argument applies to female offspring.
How does the dominant female prevent the others from laying? "The small group sizes and small physical nest size in *P. alternata* suggest that queen policing of subordinate oviposition is feasible." (19) Whether this policing behavior occurs by physical inhibition or chemical regulation remains open (see (20) for the relevance of this to the evolution of social behavior).

Placing the scene in MLS spotlights: again, what do we call the 'group'? In the previous case, with the primitively eusocial wasp *E. calyptodoma*, there were only two females cooperating for a very short time. Based on that, and on the fact that part of the reproduction took place outside of the group, I refused to call it a group. Unfortunately, information about male-male competition lacks in this article. I expect that a similar system is at work as in many other Hymenoptera; after pupation, newly emerged females are fertilized in their natal nest before dispersing (e.g. (21)). The question whether they are fertilized by their nestmates or by others remains open. Let's assume the first; can we then call the small colonies of *P. alternata* groups? The MLS **1** assumption that the group will –at least during the reproductive phase- stay together would then be met. Note that there can be a certain degree of gene-flow between these groups. Before making the final remarks, one last observation:

Figure 3
The relationship between group size (number of adult females) and total productivity. Open squares indicate sample 1 ($n = 16$), and filled circles indicate sample 2 ($n = 18$). The solid and dashed lines are the lines of best fit for samples 1 and 2, respectively.

"Group size, as the number of females on a nest when it was collected, had a significant effect on total productivity (fig.3 (19))". Although the authors interpret this result as "indicating that subordinate females gain indirect fitness benefits from helping", I would state that at least it indicates that a when a dominant female is capable of 'organizing' multiple helpers at her nest, she would certainly benefit from that in terms of reproductive success (as we saw earlier that offspring-production was nearly always monopolized by a single dominant female).

Now, we can create an overview of the different selection processes at work:

First, we can expect a certain amount of within group competition, both based on the fact that all adults are capable of reproducing and since the relatedness patterns within the nest are not as tight as in truly social species.

Second, a group containing more females has a higher productivity. They will thus have a bigger contribution to the 'overall gene-pool' from which the next generation is formed. Furthermore, the authors point out that there might be a strong ecological constraint on solitary nesting; it is imaginable that only the strongest females make it to found their own nest. Now, assuming that dominance is heritable and correlated with 'self founding' chances, I can imagine that these highly productive groups with their very dominant females produce daughters with higher self-founding changes than daughters from less productive groups. In this way, selection within the group (by competition) and between groups (by their contribution to the gene pool) will favor the policing trait within the population. As far as I can see, this would be a perfect MLS **1** setting.

Last, but not least, an expansion of the Multi-Level Selection Theatre: group density at the *colony level* might play a role in the above described mechanism, as this may influence self-founding chances. I can imagine that selection favors the 'being-selffounding-and-dominant' trait more in colonies with lower nest-densities. Off course, these different nest densities may arise from differences in ecological conditions and can thus vary both in space and time (e.g. between years but within the same colony).

Eusocial; Vespula maculifrons.

After hibernation, a single –already mated- *V. maculifrons* queen founds a new colony in early May (22). She constructs the incipient nest and rears the first cohort of workers (23). Once the workers mature, they take over the task of colony maintenance and the colony will grow rapidly (reaching a peak population of nearly 3000 workers in September (22)). *V. maculifrons* exhibits clear sexual and caste dimorphisms, with queens being much larger than both males and workers (24) (see coverpicture, (23)).

The queen remains wholly responsible for the production of all offspring within the nest as long as she is present. The workers do not mate and therefore can never produce female offspring. They only produce males if the colony becomes queenless (23). At the end of the summer, the colony begins to produce males and new reproductive queens (gynes). The colony will consist of about 8000 (!) cells by that time -of which nearly 30% produce queens (22). Gynes must be reared in specially constructed, larger cells (see coverpicture, from (23)). *Vespula maculifrons* colonies produce hundreds of gynes and males in a season. Colonies typically survive only one year so the number of sexuals produced at the end of the season represents the entire reproductive output of the colony and, by extension, the queen (23).

Upon reaching sexual maturity, both males and gynes leave the nest to partake in mating flights (24). Based on limited data, it is believed that *V. maculifrons* males lek near prominent vegetation with few competitive interactions occurring between males (25). Gynes typically mate with multiple males (23). In some vespine wasps, female-produced sex pheromones attract males from a distance and stimulate courtship behaviors such as mounting and attempts to copulate (26). Many species appear to discriminate among mates at the level of individual or kin/colony, using anti-aphrodisiacs to prevent mating with relatives (26). In an experimental set-up, Kovacs et al. failed to detect any evidence of nestmate avoidance during the mating trials of *Vespula maculifrons* (24). However, they did find that males and females from one particular colony displayed significantly greater mating activity than individuals from other colonies. They also found that larger females had a higher reproductive success. Kovacs et al. concluded therefore that the colony from which an individual originate plays an important role in determining mating success. (24)

To analyse the evolutionary processes shaping the populations of *V. maculifrons*, we have to differentiate clearly between two questions. First, we might wonder how this system with its workers who 'lack reproductive totipotentie' (27) evolved in the first place. The second set of interesting questions concern the type of selection pressures which might have played a role since workers lack the ability to become the primary breeders -and which are at work now. Let me start trying to answer this last question by looking at the colony traits described above. Defining a group is here more obvious as in the previous cases; for simplicity, I will call the queen and her co-workers (all living in the same nest) the group. Again, I can imagine processes above this group-level; selection acting differently on population scale, in which for example nest-density acts as an important factor both during the founding phase as in the reproductive phase. In which MLS-theatre would these groups fit?
In *V. maculifrons*, reproduction occurs in mating flights in which individuals from different groups participate (24). This is the most obvious reason to group this example into the concept of MLS **1**; the reproductive output of a group is shuffled again in a big gene-pool from which new groups are formed. Furthermore, although within-group competition is more silenced than in the other examples (since workers hardly reproduce), it is still apparent when the queen dies. Workers then start producing males (23), so there must be selection acting on the within-group variation, even when the produced males do not show intense competition on their 'leks' (25). And- as an extension of this argument- *V. maculifrons* queens have multiple mates, which even increases the within-group variation (23). Finally, some groups are better than others in producing gynes that are large or exhibit other sexy characters (since colony-

origin predicts mating activity (24)). And I assume that group-origin will also correlate with the absolute number of sexuals added to the gene pool.

Thus, at the moment, a system is at work in which selection pressures play different roles at different levels within the concept of MLS **1**. However, this degree of eusociality raises the question how the system evolved in the first place. Here, I will briefly review the literature about the origin of eusociality, focusing on the relevancy to the case of *Vespula maculifrons*.

As we know, the problem of social behavior lies in the question why an individual should carry out a cooperative, or helping, behavior that appears costly to perform, but benefits other individuals (10). In 1963, Hamilton showed that individuals might "gain inclusive fitness through their impact on the reproduction of related individuals (indirect fitness effects) as well as directly through their impact on their own reproduction (direct fitness effects)" (8) (10). From this, it follows that helping behaviors might have evolved in two ways; the direct fitness *benefits* of the helping behavior outweight the *cost*, or the helping provides an indirect benefit because it is directed to related individuals (see appendix for a classification of the explanations for cooperation by West et al. (8)). Clearly, these ideas contrast with the concept of MLS **2**, where cooperative/ helping traits are favored by selection through the differential reproduction and survival of groups, when these groups show different amounts of cooperation. But note that there is no fundamental difference with MLS **1;** both theories focus on the differential survival and reproduction of individuals, but MLS emphasizes that these individuals can be organized in a group-structure (e.g. (8))**.**

In 2005, E.O. Wilson wrote in a review that "eusociality arises by the superiority of organized groups over solitaires and cooperative pre-eusocial groups. It can, in theory at least, be initiated by group selection in either the presence or absence of close relatedness and, when close relatedness exists, also in the presence or absence of kin selection. Conversely, eusociality cannot arise without the driving force of group selection, regardless of the degree of relatedness within local populations or cooperative aggregations. Close kinship may be more a consequence of eusociality than a factor promoting its origin." (28)

This last statement was tested by Hughes et al. with a comparative analysis of female mating frequencies in 267 species of eusocial bees, wasps, and ants. They found "that mating with a single male, which maximizes relatedness, is ancestral for all eight independent eusocial lineages. Polyandry is always derived and occurs only in lineages whose workers have lost reproductive totipotency. Thus, monogamy was critical in the evolution of eusociality."(27)

As we have seen, *V. maculifrons* is a polyandrous species. Goodisman et al.(23) showed that "queen mate number was directly, strongly, and significantly correlated with the number of queen cells produced by colonies. Polyandry is thus adaptive since it provides fitness advantages to queens." However, the findings of Hughes et al. will also hold for the ancestors of *V. maculifrons*. Thus, in earlier times, monogamous queens reared colonies consisting of highly related sisters. Daughters gained indirect fitness benefits when helping their mother to raise new daughters. Colonies, or 'groups', containing more helpers could probably deliver more offspring to the overall gene pool; we can therefore conclude that the eusociality levels of *V. maculifrons* are probably evolved in the MLS **1** setting.

Conclusion & Discussion

The investigation of the different levels of sociality in the colonies of different species within the family *Vespidae* made a couple of things more clear. First, I showed that the definition of what we will call a 'group' is not as tight as one might expect when looking at the comparison from West et al. (8). Maybe more important is that for each separate case investigated, I showed how selection can act on different levels. Summarizing; in all the cases considered, a certain amount of competition between individuals was observed –resulting in selection on individual traits. As an extra level of selection, the structuring of the individuals into certain groups played an important role. In the first two cases nest density affected reproductive decisions (*E. foraminatus* changed brood investment and in *E. calyptodoma* usurption changes changed). In the last two cases, the structuring of the individuals at the *nest-level* had clear evolutionary consequences. In *P.alternata*, group productivity increased with group size, resulting in differential contributions to the gene-pool. And also in *V. maculifrons*, group origin correlated with offspring quality (and I assume quantities). Furthermore, we could speak of an extended version of MLS **1** in these last two examples, since the density of *groups* within a certain *population* might reinforce the selection pressures acting on group-productivity.

Overall, I conclude that the concept of Multi-Level Selection type **2** is not applicable to any of the cases. I have to point out, however, that some species the strict assumptions of MLS **2** might be easier met: these colonies are truly bounded groups with very low migration rates between them, they do produce 'daughter-groups' when a part of the colony splits up and establish somewhere else.

As I have explained in the introduction, selection could act on such a system when the group-traits meet the Darwinian principles of heritability, variation and reproductive succes. In 1988, Damuth and Heisler (5) created a classification of characters, or traits, on which selection could act (see appendix). They stated that "the theoretical unity of the multilevel selection traditions becomes more apparent when it is recognized that the different approaches do not advocate mutually exclusive fundamental processes or different evolutionary theories. Neither MLS 1 nor MLS 2 is the sole legitimate approach to multilevel selection. Rather, they represent two aspects of the increased complexity residing in any multilevel selection situation."

But can the group traits they propose –the population mean of a trait, or a population density- be subject to selection? I hope that I have clarified with this thesis that it depends highly on the study system whether one focuses more on the selection processes acting on individuals, groups or populations. The question whether the group-traits as suggested by Damuth and Heisler are useful definitions for a process like MLS **2** to act, are only of conceptual value. So, although I think that their way of defining group-traits does no justice to the concept of MLS **2,** I agree that these traits add to the complexity of multi-level selection processes.

During the analysis of *Vespula maculifrons*, I referred to E.O. Wilson's statements on how eusociality had evolved. He pointed towards to the importance of group selection as a distinct mechanism from kin-selection. But carefully reading his article shows that the group-selection mechanism to which he is referring is actually the MLS **1** situation (groups add their reproductive output to a larger gene-pool). Again, I'd like to point out that there is no need to make a choice between these mechanisms; in different situations, the balance between the within-group mechanisms and the fact that the individuals are structured into groups might play different roles.

Although the concept of MLS **2** wasn't applicable to any of the described cases, I do not state that the process itself is useless. Still, I think it could be at work in nature under the –maybe very extreme- conditions of absent migration and intense between-group competition.

The debates on the concept of MLS **2** grew vividly again during the last decennia, in which the idea grew that within-group mechanisms may exist that counteract the between-group-variation-eroding forces of within-group competition and migration. These mechanisms might play an important role in the evolution of *human* sociality and are all based on the idea that evolution can act not only on genetic information, but also on cultural information (examples of these mechanism are 'conformist biases', 'blending' and 'cognitive attractors'). Some people state that these mechanisms also provide a solution to the question how a group-trait might be heritable. Unfortunately, it is beyond the scope of this research to go into the details of these processes, but see (29) (30) (31). But I have to point out here, when looking at the intensity of these Cultural Inheritance debates (e.g. (29)), that fixating at the difference between these two Multi-Level Selection processes does not bring more clarity. As we have seen, complex Multi-Level Selection situations can arise in different cases. Focusing on the case of interest and unraveling the mechanisms step by step –using comparative approaches (27) and experiments (24)- might bring us closer to "the Truth".

Front cover "**The Social Biology of Wasps**"
K.G. Ross & R.W. Matthews. (*E. calyptodoma*?)

Acknowledgements.

References

1. **Darwin, C.** *The origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London : John Murray, 1876.

2. **Wynne-Edwards, V.C.** *Animal Dispersion in Relation to Social Behavior.* Edinburgh, Scotland : Oliver & Boyd, 1962.

3. *Group Selection and Kin Selection.* **Maynard Smith, J.** 4924, March 14, 1964, Nature, Vol. 201, pp. 1145-1147.

4. *A Theory of Group Selection.* **Wilson, D.S.** 1, January 1975, Proc. Nat. Acad. Sci. USA, Vol. 72, pp. 143-146.

5. *Alternative formulations of multi-level selection.* **Damuth, J., Heisler, I.L.** 1988, Biology and Philosophy, Vol. 3, pp. 407-430.

6. *Alternative Formulations of Multilevel Selection.* **Damuth, J., Heisler, I.L.** 3, s.l. : KluwerAcademic Publishers, 1988, Biology and Philosophy, pp. 407-430.

7. *Maynard Smith and the level of selection question.* **Okasha, S.** s.l. : Springer, May 3, 2005, Biology and Philosophy.

8. *Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection.* **West, S.A., Griffin, A.S., Gardner, A.** 2007, European Society for Evolutionary Biology, Vol. 20, pp. 415-432. doi:10.1111/j.1420-9101.2006.01258.x.

9. **Dawkins, R.** *The Extended Phenotype: The Long Reach of the Gene.* Oxford : Oxford University Press, 1982. over longevity, fecundity, fidelity.

10. *The evolution of altruistic behaviour.* **Hamilton, W.D.** 1963, American Naturalist, Vol. 97, pp. 354-356.

11. *The Evolution of Eusociality.* **Ansdersson, M.** 1984, Annual Review of Ecology and Systematics, Vol. 15, pp. 165-189.

12. **Wilson, E.O.** *The Insect Societies.* Cambridge, Massachusetts : Harvard University Press, 1971.

13. *The definition of eusociality.* **Crespi, B.J., Yanega, D.** 1, 1994, Behavioral Ecology, Vol. 6, pp. 109-115.

14. *Social terminology revisited; where are we ten years later?* **Costa, J.T., Fitzgerald, T.D.** Helsinki : s.n., December 21, 2005, Ann. Zool. Fennici, Vol. 42, pp. 559-564.

15. *Simultaneous Analysis and the Origin of Eusociality.* **Pickett, K.M., Carpenter, J.M.** 1, February 28, 2010, Arthropod Systematics & Phylogeny, Vol. 68, pp. 3-33.

16. *Parental investment in two solitary wasps Ancistrocerus adiabatus and Euodynerus foraminatus (Eumenidae: Hymenoptera).* **Cowan, D.P.** 2, September 1981, Behavioral Ecology and Sociobiology, Vol. 9, pp. 95-102. 10.1007/BF00293580.

17. *Elements of eusociality in colonies of Eustenogaster calyptodoma.* **Hansell, M.** February 1987, Animal Behavior, Vol. 35, pp. 131-141.

18. **terraincognita96.** *www.flickr.com.* [Online] September 5, 2009. [Cited: July 31, 2010.] http://www.flickr.com/photos/29697818@N03/3890881124/in/set-72157621991649340/.

19. *Colony genetic structure in a facultatively eusocial hover wasp.* **Bolton, A., Sumner, S., Shreeves, G., Casiraghi, M., Field, J.** July 13, 2006, Behavioral Ecology, Vol. 17, pp. 873-880. doi:10.1093/beheco/arl020.

20. *Ropalidia marginata – a primitively eusocial wasp society headed by behaviourally non-dominant queens.* **Sumana, A., Raghavendra Gadagkar.** 11, June 10, 2003, Current Science, Vol. 84, pp. 1464-1468.

21. *Comparison of spermatheca morphology between reproductive and non-reproductive females in social wasps.* **Gotoh, A., Billen, J., Hashim, R., Ito, F.** 2008, Arthropod Structure & Development, Vol. 37 , pp. 199-209. doi:10.1016/j.asd.2007.11.001.

22. *Nesting Biology of the Eastern Yellowjacket, Vespula Maculifrons (Hymenoptera: Vespidae).* **MacDonald, J.F., Matthews, R.W.** 3, July 1981, Journal of the Kansas Entomological Society, Vol. 54, pp. 433-457. only abstract available. http://www.jstor.org/stable/25084177.

23. *The significance of multiple mating in the social wasp Vespula maculifrons.* **Goodisman M.A.D., Kovacs J.L., Hoffman E.A.** 9, September 2007, Evolution, Vol. 61, pp. 2260-2267 . ISSN: 0014-3820 .

24. *Mating Success in the Polyandrous Social Wasp Vespula maculifrons,.* **Kovacs, J.L., Hoffman, E.A., Goodisman, M.A.D.** 4, 2008, Ethology, Vol. 114, pp. 340-350. doi: 10.1111/j.1439-0310.2008.01487.x.

25. **Ross, K. G., Carpenter, J. M.** Population genetic structure, relatedness, and breeding systems. [book auth.] K. G., Matthews, R.W. Ross. *The Social Biology of Wasps.* Ithaca, NY : Comstock publishing associates, 1991, pp. 451—479. viewed in Google books.

26. *Mating Behavior and Chemical Communication in the order Hymenoptera .* **Ayasse, M., Paxton, R.J., Teng, J.** 2001, Annual Review of Entomology., Vol. 46, pp. 31–78.

27. *Ancestral Monogamy Shows Kin Selection Is Key to the Evolution of Eusociality.* **Hughes, W.O.H., Oldroyd, B.P., Beekman, M., Ratnieks, F.L.W.** 2008, Science, Vol. 320, p. 1213. DOI: 10.1126/science.1156108.

28. *Eusociality: Origin and consequences.* **Wilson, E.O., Hölldobler, B.** 38, September 20, 2005, PNAS, Vol. 102, pp. 13367–13371.

29. *Five Misunderstandings About Cultural Evolution.* **Henrich, J., Boyd, R., Richerson, P.J.** 19, 2008, Hum Nat, pp. 119-137.

30. **McElreath, R.** The coevolution of genes, innovation and culture in human evolution. [book auth.] J. Silk & P. Kappeler. *Mind The Gap: Tracing the Origins of Human Universals.* s.l. : Springer, 2010, pp. 451-474.

31. *For the Good of the Group? Exploring Group-Level Evolutionary Adaptations Using Multilevel Selection Theory.* **O'Gorman, R., K.M.Sheldon, D.S.Wilson.** 1, 2008, Group Dynamics: Theory, Research, and Practice, Vol. 12, pp. 17–26. DOI: 10.1037/1089-2699.12.1.17.

32. *Reproductive constraints, direct fitness and indirect fitness benefits explain helping behavior in the primitively eusocial wasp, Polistes canadensis.* **Sumner, S., Kelstrup, H., Fanelli, D.** February 3, 2010, Proceedings of the Royal Society B, Vol. 277, pp. 1721-1728. doi:10.1098/rspb.2009.2289.

Appendix - "something attached".

Solitary wasps *A. adiabatus* & *E. foraminatus.*

"Under conditions of clumped nesting, females make larger offspring and invest more in males than when nesting in isolation" (16)

Table 2. Effect of isolation and aggregation of nesting females on patterns of parental investment

Nesting females	Sons		Daughters		Investment ratio
	Mean provision weight (mg)	*n*	Mean provision weight (mg)	*n*	
Ancistrocerus adiabatus					
Isolated	50	238	83	787	0.15
Clumped	53	48	84	129	0.19
Euodynerus foraminatus					
Isolated	124	610	209	520	0.41
Clumped	136	114	233	87	0.43

Damuth & Heisler on MLS; individual versus group traits.

Group characters are divided into aggregate-type characters and global characters.

TABLE 1
A Classification of Characters. (After Lazarsfeld and Menzel, 1961)

Character-type	How measured	Examples
Kinds of Characters Assignable to Individuals:		
Individual	Measured directly on individuals	Any measurement or quality of an individual
Contextual	Measured on groups; assigned to individuals; may be *any* kind of group character (see below)	Group mean; population density of the group; the proportion (frequency) of different phenotypes in the group
Kinds of Characters Assignable to Groups:		
Aggregate	Obtained by combining measurements on individuals	Group mean; the proportion (frequency) of different phenotypes in the group
Global	Not obtainable by measuring individuals; "emergent" characters	Population density

West et al. on Cooperative Behavior.

A classification of the explanations for cooperative behavior (central problem: why help?)
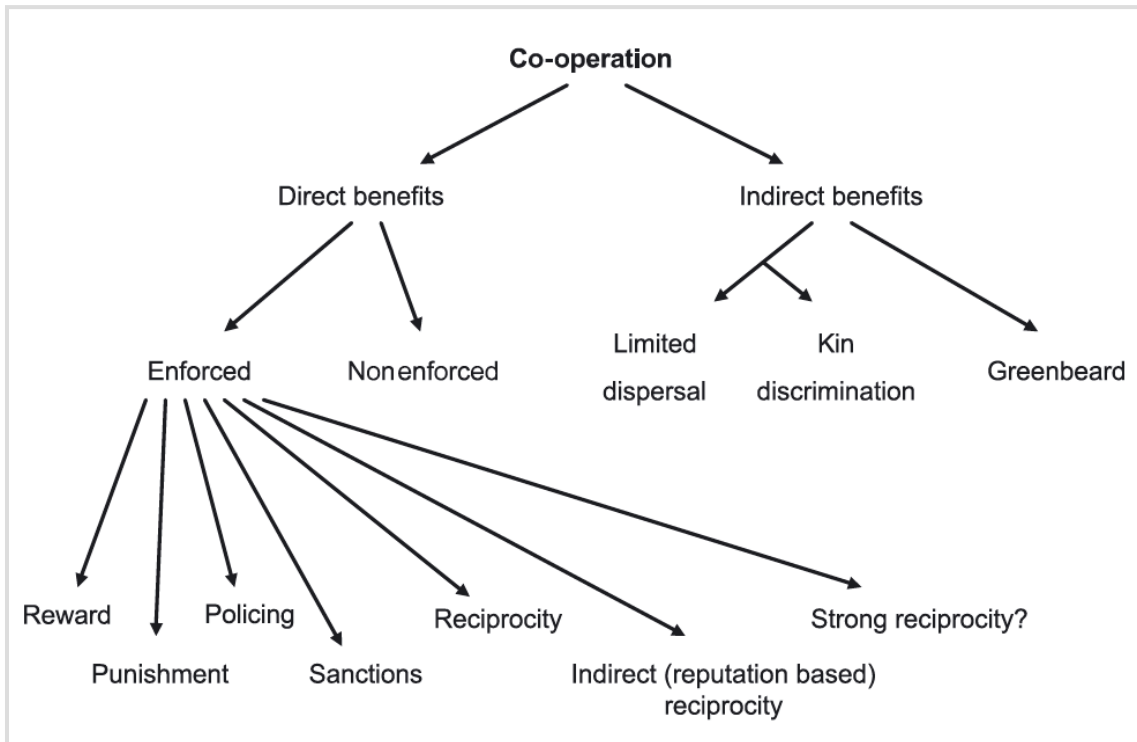

Fig. 1 : A classification of the explanations for cooperation.
Direct benefits explain mutually beneficial cooperation, whereas indirect benefits explain altruistic cooperation. Within these two fundamental categories, the different mechanisms can be classified in various ways – here we follow West et al. (2006; see also Sachs et al., 2004; Lehmann & Keller, 2006). These possibilities are not mutually exclusive, for example a single act of cooperation could have both direct and indirect fitness benefits. We have listed some of the many different terms that have been used to describe the mechanisms for enforcing cooperation to emphasize that reciprocity (reciprocal altruism) is only one of many ways to obtain direct fitness benefits through cooperation. These enforcement mechanisms can also alter the indirect benefits of a behavior (Lehmann & Keller, 2006), and determining the relationships between these terms remains an important task. Kin selection has been used to refer to (i) just those indirect benefits involving coancestry (i.e. limited dispersal and kin discrimination), or (ii) all indirect benefits (i.e. also including greenbeard effects).