The mechanisms and genetic basis of immunity trade-offs

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Abstract

The trade-off between the immune system and other traits has been studied in some detail. This trade-off is particularly interesting because it could explain the great amount of variation in the expression of traits among individuals. Especially insects are attractive for studying the immune system because their lack of the complex acquired immune system. About the mechanism underlying the trade-off, however, much remains unknown. Recent genetic studies already gave more insight about the genetic basis underlying the mechanism of a trade-off. In this review mechanisms underlying trade-offs between the immune system and other traits found by studies are compared to see if they have a similar basis or are completely different. With the use of genetic studies there will be looked at which evidence is available for the genetic basis underlying the trade-off. It seems that trade-offs between the immune system and other traits are mainly due to resource allocation controlled by a switch like the insulin-receptor pathway. However, trade-offs between the immune system and other traits could also be due to the fact the immune system is harmful for the organism itself.

Content

Introduction

The life of an organism is an complex interplay of many traits that together determine the survival and life reproductive success of an individual. Traits can be studied in different ways, separate or compared amongst each other. When comparing traits to each other, some traits seem to be negatively associated (Rolff 1992). Because of this negative association there is a great variation in the expression of certain traits. Immunity is a particulary interesting trait in this setting, because it shows large variation among individuals and has a great influence on the survival of an organism but also on the reproductive success (Schmid-Hempel 2003).

A trade-off gets visible when internal resources are limited and are insufficient to pay all construction and maintenance costs for two life history traits that share a common resource gene pool (Zera & Harshman 2001). An increase of resources for one trait will be balanced by a decrease of resources for an other trait. A trade-off can be defined by using a genetic model, such as a complex version of the Y-model. If internal resources are limited and two life history traits share a common source pool a trade-off is the result. An increase of resources for one trait simultaneously decreases resources for the other trait (Zera & Harshman 2001). Alternatively, a trade-off can be defined using a model that selects on a certain trait which is accounting for fitness and measures correlated declines in other traits. The latter is called the optimality approach (Rolff 1992) and is based on the assumption that an organism optimizes his trade-off to the environment. It is important to see that an organism is optimizing his trade-off rather then maximizing it. It is not always the case that when traits are negatively associated there is a trade-off. It could be due to genetic linkage (Zera & Cisper 2001). The alleles that determine the expression of traits can be physically close on the chromosome and hereby inherited together. When the allele for strong expression of one trait is always inherited with an alle for weak expression of trait, it is logic to see a negatively association.

The nutrient input is a important factor in a trade-off, and when studying trade-offs the nutrient input should be controlled of quantified (Rolff 1992). The timing between nutrient acquisition and the trade-off is an important aspect. For many organisms there is a time gap between nutrient input and the eventual outcome of a trait (like reproduction). In capital breeders; who store their energy for later use in reproduction; the timing of the trade-off is important, otherwise the energy stored for a certain trait is already used (Zera & Harshman 2001). Nutrient acquisition is a known factor to influence a trade-off, but there are also many non-energetic factors that can influence trade-offs, for example hormonal control of antagonistic traits (Zera & Harshman 2001). This is why trade-offs are dynamic and not static, and why they can change during development and can evolve (Leroi *et al*. 1994). An example of a static trade-off is that the in the early stages of the life of an organism more energy is spent on development, but in the late stages of an organism it is the other way around.

A well studied trade-off is the trade-off between the immune system and other traits. When the immune system is activated and maintained this is costly for other traits, such as reproductivity (Sheldon & Verhulst 1996). The study on bighorn ewes (*Ovis canadensis*) showed that lactating individuals had greater faecal counts of lungworm than non-lactating individuals (Festa-Bianchet 1989). This difference suggested that the lactating bighorn ewes did not have the same resources available for parasite defense as the non-lactating bighorn ewes. The trade-off between the immune system and other traits has now become a big study area (Zuk & Stoehr 2002), it could explain the great variation of expression of traits among individuals (Sheldon & Verhulst 1996).

The immune system can be divided in two different parts: the innate immune system and the acquired immune system. The innate immune system is always present and can be activated by any diseases (pathogens) in the environment, while the acquired immune system is adapting to and remembering specifically specific challenges (Zuk & Stoehr 2002).

Recently there has been a shift to studying invertebrate immunity. A major reason for this shift is that invertebrates are attractive subjects to study because of the relatively simple mechanisms used by their innate immune system (Rolff & Siva-Jothy 2010). Studies are hoping to find a genetic basis of the trade-off between the immune system and other traits. Some studies already found evidence with a negative correlation within different parts of the immune system (Cotter *et al*. 2004). When trade-offs are found between the immune system and an other traits this genetic information may help to understand the trade-off in an evolutionary context.

Studying a trade-off requires a broad knowledge of your organism. To cover all the aspects of trade-offs, one must look at ecology, metabolism, immunology and genetics of the studied organism. Not every study covers these different areas, and hereby many results of studies remain unexplained. Often a study finds a trade-off but can't explain the mechanism of how it is working. This review will try to find mechanisms responsible for trade-offs between the immune system and other traits in invertebrates and relate this with genetic research in this area. Are the mechanisms completely different from each other or is there a similar basis across various trade offs? What evidence is available for the genetic basis underlying the mechanism of the trade-off? I will restrict my review to insects, because the immune system of insects is well studied and relatively simple in comparison with the immune system of vertebrates.

The immune system in insects

To understand trade-offs between the immune system and other traits, knowledge about how the immune system works is required. I will give a summary of the immunity of insects. The immune system of insects has mostly been researched by using *Drosophila melanogaster* as a model organism (Schmid-Hempel 2004). Insects can defend themselves by behavior or the immune system.

Defense by behavior is the first line of defense (Hart 1997). Behavioral defense could be an effective response to selection from high-cost parasites or the cost of a pathogen insult, to avoid infestation. Many parasites will enter the insect via the host's meal, so there could be a selection on a particular way of foraging by which the insect doesn't consume parasites. Also, mate-calling behaviour may be adjusted to minimize the risk of detection by parasites (Siva-Jothy *et al*. 2005). If there is such a selection parasite defense could be responsible for particular behavior, but this is not well studied (Siva-Jothy *et al*. 2005).

The second line of defense is the immune system which could be grouped by a boundary defense and the haemocoelic defense. The boundary defense is the outer body covering. It consists of a toughened cuticle that protects the insect's external surface. Although this is a rigid barrier to the outside world, there are weak points that could be targeted by a parasite or pathogen. The epidermis lying under the cuticle is a site of the expression of key immune effector systems and in this way it plays also an important role in the defense (Siva-Jothy *et al*. 2005).

Once an intruder has succeeded to break through the boundary defense it encounters the haemocoelic defense of the insect. The haemocoelic defense produces a rapid and effective response that localizes and neutralizes the reproductive capacity of the pathogen or growth potential of the parasite. Insects don't have an acquired immune defense so they will be relatively indiscriminatory when confronted with subtle different types of non-self. Insects have an open haeomocel, in contrast to a closed circulatory network in vertebrates, which has some advantages as well as disadvantages in terms of functioning of the immune system. Because of the open haemocoel, the reactive products of the immune system can be

transported more rapidly, but this is also the case for infective agents (Siva-Jothy *et al*. 2005). So it is important that when there is a wound, it must be dealt with rapidly. The wound gets healed by clotting: the haemolymph gets an increased viscosity and there is a formation of glycolysated fibers that will seal the wound (Scherfer *et al*. 2004).

The insect immune system can recognize non-self, and needs to avoid reacting to self. Organs and tissues in the haemolymph are covered with a basal lamina which is there to provide a good sign of self. Hereby non-self particles will be recognized and treated as more suspicious. The non-self particles are identified against this self background by pattern recognition peptides. When the non-self particles have been identified, this has to be signaled to an immune response, which can produce effector molecules that target the non-self particles. There is humoral-based signaling and cell-based signal transduction pathways. One of the most important humoral-based effector system is prophenoloxidase (Gorman & Paskewitz 2000). The cell-based signal transduction pathways are named after key proteins that mediate the signal: Toll (a transmembrane receptor) and Imd (an intracellular signalling molecule). Activation of the Toll pathway results in the synthesis and secretion of antifungal peptides and peptides that act against bacteria and fungi (Siva-Jothy *et al*. 2005), as well as the activation of haemocytes (Qiu *et al*. 1998). When the Imd pathway gets activated it induces the regulation of antimicrobial peptides against gram-negative bacteria (Hultmark 2003).

Through the activation of signaling pathways effector systems are activated. This can be in the form of enzyme cascades and cytotoxins, antimicrobial peptides and haemocytes. Phenoloxidase is an important cytotoxin, it produces the biopolymers melanin and sclerotin by which it externalizes the intruder. Haemocytes can perform phagocytosis on pathogens smaller than themselves. When the target is too big for a haemocyte to perform phagocytosis it will stick onto the target with other haemocytes and in this way smother the target. When the target is surrounded by a layer of haemocytes it will be melanized, a process in which the cytotoxin phenoloxidase has a big role (Siva-Jothy *et al*. 2005). Some insects have parasites inside their body, called parasitoids. There parasitoids will be inoculated as egg and when they hatch kill the host insect (Kraaijeveld *et al*. 2000). Hereby the process of melanisation is essential in killing the parasitoid, otherwise the insect will not survive. In this way there is a great selection pressure on completing this response with success.

Having the immune system and using it is not without costs. Life history theory assumes that the immune system has a cost that is traded-off with other traits (Sheldon & Verhulst 1996). The costs of the immune system can be separated into evolutionary, maintenance and deployment costs, that differ in timescale and implications (Schmid-Hempel 2003).

Evolutionary costs are costs that relies on a negative association two different traits. The variation of the expression of the immune system influences the expression of other traits. Over time this can be genetically fixated and the individual can not change this trade-off. Yet, the maintaining of the immune defense is still a plastic trait (Siva-Jothy *et al*. 2005). There will be an evolutionary cost when the immune system will develop itself over time due to expenses on another trait (Schmid-Hempel 2003).

The maintenance of the immune system keeps the immune system on a certain level of alertness, hereby it can act quickly when an action is required. The deployment of the immune system is the actual action that is performed when there is a challenge. The maintenance of the immune system is probably maintained by trade-offs and is difficult to measure because it may interact with different other regulatory processes. This is why the deployment costs are more often measured (Schmid-Hempel 2003). When challenging the immune system in a certain way and measuring the changes in the immune system, traits in comparison to controls

can show the effect of the deployment of the immune system. However in studies where the effects of deployment are measured it is difficult to separate these costs from the actual damage that the pathogen or parasite caused (Kraaijeveld *et al*. 2002). To prevent measuring the damage of parasites,some studies use particles that are not harming for the individual itself, like Sephadex beads (Koella J.C. & Boëte C. 2002). But because non-harming particles don't damage the organism, it could be that the immune system is less activated in comparison with a parasite.

Trade-off between the immune system and the reproductive system

Many studies have found evidence for an trade-off between the immune system and the reproductive system (Ahmed *et al*. 2002; Schwartz & Koella 2004; Zhong *et al*. 2005; Gwynn *et al*. 2005).

A study by Gwynn *et al.* 2005 provided evidence for a trade-off between the ability to resist a parasitoid attack and fecundity in the pea aphid. Gwynn *et al.* (2005) used clones of pea aphid that were parasitized by the wasp *Aphidius ervi* and registered the resistance of the pea aphid to the parasitoid, where after the fecundity of the clones was measured. The authors found evidence for a trade-off between the resistance against a parasitoid attack and fecundity. The evidence that was found for a trade-off between resistance and fecundity is primarily the result of the loss of early fecundity. When looking at the late fecundity there seems to be no evidence for a trade-off (Gwynn *et al*. 2005). In the pea aphid there is a primary obligate mutualistic endosymbiont that has been shown to have an direct additive effect on the pea aphid's fitness. But there are also secondary symbionts which could contribute to an increased parasitoid resistance. The physiological basis for this increased parasitoid resistance due to secondary symbionts is poorly understood (Oliver *et al*. 2003). Possibly, the secondary symbiont has costs in terms of nutrients, but this could be counter-balanced by the beneficial effects of the increased resistance. When the rate of infestation is high in the environment, there should be more benefit to an increased resistance. However, when the rate of infestation is low, having to miss nutrients due to the secondary symbionts for an increased resistance is costly.

The data of a study with the mosquito *Anopheles gambiae* showed that immune stimulation resulted in the allocation of fewer resources for reproduction (Ahmed *et al*. 2002). The humoral immune system of the mosquito *A. gambiae* was challenged by the injection of grampositive bacterium. The immune challenged flies had a lower production of eggs in comparison with the controls. Ahmed *et al. 2002* suggests that there could be direct competition between resources for reproduction and immune defense, but only when there are limiting factors,such as resource limitation. An alternative explanation the authors give could be via 'danger signals' that are inducted via an immune response. These signals could directly or indirectly have an affect on the egg production and reduce it. But there was no correlation found between the degree of immune response and fecundity reduction, by which a trade-off due to resource allocation seems not very confident. It could be the case that the defense response is in itself harmful to the mosquito by which its leads to egg reduction without a direct link or resource limitation.

In a study of Schwartz & Koella (2004) the mosquito *Aedes aegypti* was inoculated with Sephadex beads with different conditions to see the encapsulation response. They used a negatively charged Sephadex bead and a neutral Sephadex bead. There was only a cost observed in the negatively charged bead. When this bead was melanized, the costs what could be seen in the number of eggs produced was reduced by a factor of 2. The neutral Sephadex bead did not show any cost to fecundity although it was melanized. This bead-dependent cost

could give some insight in the process underlying the cost (Schwartz & Koella 2004). This study did, however, not find evidence for a trade-off between the immune system and fecundity. When mosquitoes received a larger blood meal, their immune defense was be more efficient. Hereby there will be a higher cost due to the melanisation response, but the authors did not found a correlation between blood meal size and costs. The reason that the neutral Sephadex bead did not gave costs could be due to different resource allocation between different immunogens (Schwartz & Koella 2004).

In a field experiment Jacot *et al.* (2004) manipulated the nutritional condition of male field crickets *Gryllus campestris* and investigated the effect of an induced immune response through inoculation with bacterial lipopolysaccharides. They found that an immune insult caused a lasting reduction in sexual display and longevity. However food supplementation improved the longevity and sexual display, the cost of immunity was indepent of the nutritional condition of the field crickets. The trade-off between investment in immunity and male sexual display concerned limited energy reserves (Jacot *et al*. 2004). Immune respons in insects are costly, a calling male field cricket has a three times higher metabolic rate then a non-calling male field cricket. This would suggest that there is a physiological trade-off with the investment in the immune system and sexual display. But apparently this cost cannot be repayed by food supplementation.

There are several studies suggesting a trade-off between immunity and reproductivity, although some studies did not find evidence for such a trade-off. The mechanism that most studies found is mostly based on resource allocation and nutrition. Gwynn *et al.* 2005 found a loss of early fecundity in the pea aphid when challenging the immune system. This could be due to the cost of the secondary symbiont. The primary symbiont has been shown to have an direct additive effect on the pea aphid's fitness, this additive effect could be decreased due to the competition between the secondary symbiont that increases resistance and the primary symbiont. In a study of Jacot *et al.* (2004), there seem to be a physiological trade-off between the investment in immunity and sexual display, which could be partially rescued by nutritional supplements. However, the cost of immunity was independent of the nutritional condition of the field crickets. This is why Jacot *et al.* (2004) suggests a trade-off concerned limited energy reserves. Ahmed *et al.* (2002) who induced a humoral immune response in mosquitoes suggests that the defense is in itself harmful for the mosquito by which its leads to egg reduction without a direct link or resource limitation. Particular components of the humoral immune response could act harmful upon the individual itself. The other mosquito studie of Schwartz & Koella 2004 challenged mosquitoes with a Sephadex bead. Schwartz & Koella (2004) did not found evidence for a trade-off between the immune system and fecundity. This could be due to the fact that the maintenance of haemocytes could not be the mechanistic basis for the trade-off because melanisation of a particle is performed directly without the layering of haemocytes. An alternative explanation could be the fact that the authors used Sephadex bead to challenge the immune system instead of a pathogen. A pathogen can be harmful for its host and hereby induce a greater immune respons than a Sephadex bead.

Trade-off between the immune system and development

There have been studies that looked at a trade-off between immunity and larval competitiveness. The studies of Kraaijeveld *et al.* (1997, 2000) and Fellowes *et al.* (1998) looked at the costs for parasitoid resistance by encapsulation. In the studies of Kraaijeveld *et al.* (1997, 2000) the egg laying parasitoid *Asobara tabida* was reared on *Drosophila*

melanogaster to select for improved counter defenses against cellular encapsulation, which is the defense of the host against parasitation. In the lines that were selected for a higher parasitoid resistance there was approximately twice the density of haemocytes in comparison with the control lines. The observation of the larvae showed that these higher resistance lines had a lower ingestion speed and a reduced survival in a high-competition environment. Fellowes *et al.* (1998) showed that larvae selected for increased resistance are associated with lower survival under conditions of food scarcity and intraspecific competition. Kraaijeveld *et al.* (2000) suggests that there may be a switch in the general energy budget of the fly from investment in feeding efficiency to the synthesis of haemocytes. This trade-off may occur during the morphogenesis of the fly. The head musculature involved in feeding and the haemopoietic organ from which haemocytes are developed, are both originating from the mesoderm of the head (Tepass *et al*. 1994). It could be that due to a trade-off during development one particular area develops more mature at the cost of the other area due to resource allocation.

A study of Koella & Boëte (2002) shows that when selecting the mosquito *Aedes aegypti* for later pupation, the body size became larger and there was a higher level of immunocompetence. The mosquito was challenged by an encapsulation response through a negatively charged Sephadex bead. The authors suggest that the three traits; later pupation, larger body size and higher level of immunocompetence; are genetically correlated. An increased level of immunocompetence could increase the chance of survival but it will also bring a slower development due to this genetic correlation. Mosquitoes that develop more rapidly allocate more resources for growth than slower developing mosquitoes, and correspondingly fewer resources are available for immunocompetence (Koella & Boëte 2002).

The studies of Kraaijeveld *et al.* (1997, 2000) and Fellowes *et al.* (1998) found that larvae of *D. melanogaster* selected for increased resistance had a lower ingestion speed, lower survival under conditions of food scarcity and a reduced survival in a high-competition environment. Kraaijeveld *et al.* (2000) suggests that there could be different trade-offs in different stages of the fly. In the larval stage the costs for developing and maintaining a high resistance will reduce the resources for development. In this way a larvae could develop a slower ingestion speed. Koella & Boëte (2002) looked at the mosquito *Aedes aegypti.* The authors found that the mosquito *Aedes aegypti* can develop fast, be small and have a weak immunity or develop slow, be big and have a strong immunity. So there seems to be a trade-off during the development stage of the fly between resources for growth and immunity. In the field, the circumstances of the environment will favor a particular the distribution of resources among these three traits that is the most optimal.
However the trade-offs of studies with *D. melanogaster* and *A. aegypti* both seem to occur during the developmental stage, the effects are different. This could be due to the fact the organisms have a totally different ecology by which certain traits are more favored.

Trade-off between the immune system and longevity

The environment is part of determining how long an organism lives. The organism adapts itself by the expression or suppression of certain traits to optimize itself to the environment. When a certain environment is more challenging than the other, you would expect higher costs for the organism and hereby the longevity decreases in comparison to a less challenging environment. What also playes a role is that an immune response could be harmful itself (Ahmed *et al.* (2002), this could also have an effect on longevity.

When an immune challenge has high costs, one could expect to see an effect on longevity. There are several studies that induced an immune response in an organism, and measured the effects on longevity (Tepass *et al*. 1994; Moret & Schmid-Hempel 2000; Armitage *et al*. 2003; Jacot *et al*. 2004).

The study of field crickets by Jacot *et al.* (2004) showed a possible trade-off between investment in immunity and longevity. They found that an immune insult caused a lasting reduction in sexual display and longevity. However food supplementation prolonged the life span, the costs of the immune insult were independent of the nutritional condition of the field crickets. The authors suggested that the reduced longevity reflected the energetic costs of a systemic up regulation of the immune system.

Bumblebee workers were challenged in a study by Moret & Schmid-Hempel (2000) with lipopolysaccharides and micro-latex beads to induce their immune system under starvation. Due to the immune activation bumblebee workers who were challenged starved earlier then workers who were not challenged. The authors concluded that under resource limitation a survival cost is continuously paid to keep infections in check. In this study a possible mechanism underlying the trade-off was not suggested.

In a study of the mealworm beetle there were deployment costs due to a immunity response albeit *ad libitum* feeding conditions (Armitage *et al*. 2003). The mealworm beetles were challenged to an encapsulation response by the insertion of a nylon monofilament. Hereby the longevity was significant reduced in comparison to the controls. The authors suggested that due to the active haemocytes playing a role in the encapsulation response there are less haemocytes available for an other immune challenge in this way the immune system is compromised. What also can contribute to the results is that when haemocytes age they may lose their function (Kurtz 2002). The combination of these two effects may mean that older, challenged beetles are less able to maintain cell-based immunity than younger and/or unchallenged beetles (Armitage *et al*. 2003). In nature, *ad libitum* feeding conditions may be rare and food-limiting conditions could mean that the differences between challenged beetles and unchallenged beetles would be more pronounced.

Multiple studies found evidence for a trade-off between immunity and longevity. However, the mechanisms behind this trade-offs where not always uncovered. The studies of Jacot *et al* (2004) and Moret & Schmid-Hempel (2000) showed costs while manipulating the nutritional condition or by starvation, in these studies there seems to be a trade-off due to resource allocation. Jacot *et al.* (2004) suggests that there are costs due to a systemic up regulation. However, food supplementation did had an influence on the costs of the immune response, it did not took the costs away. Hereby, it seems that there is a trade-off due to resource allocation. In the study of Moret & Schmid-Hempel (2000) the longevity of bumblebee's decreased under starvation when their immune system was challenged. There could be a trade-off between the use of resources to survive and the use of resources for the immune system. But when this isn't the case, it could also be that inducing the immune system is harmful to the bumblebee itself. Interestingly Armitage *et al.* (2003) did not found a trade-off due to resource allocation in the mealworm beetle. Because *ad libitum* feeding conditions still showed costs for an immunity response. This could also suggest that the immune system is harmful itself for the mealworm beetle.

The trade-off on a genetic level

Many studies have suggested mechanisms for the trade-off with immunity, but not many studies describe the mechanisms underlying trade-offs on a genetic level. With the growing

genetic tool box, more traits can be explored on genetic level. Libert *et al.* (2006) studied the impact of immune activity and function on aging and other vital characteristics. They used a GeneSwitch system of transgenic expression to activate immune pathways in a spatially and temporally controlled way. In this way the fly activates the mechanisms that are normally needed when encountering an immune challenge. The immune signaling networks were activated by overexpression of the proteoglycan protein PGRP-LE in the fat body of an adult *Drosophila melanogaster.* Activation appears to occur the most via the Imd pathway (Libert *et al*. 2006). The NFκB-related transcriptional factors activates among others the Imd pathway. Liberte *et al.* (2006) showed that acute immune system activation did not have a deleterious effect on behavioral or physiological characteristics, such as eggs per day per fly. Chronic activation that caused a chronic inflammatory condition, however, did result in a reduction of lifespan. The decreased lifespan was not the cause of a more rapidly aging immune system, because pathogen resistance in old flies in which the immune system was chronic activated was greater then identically aged flies in which the immune system did not was activated. The authors suggested a trade-off between resistance and longevity mediated by chronic NFκB signaling. The physiological cost created by the chronic activity of the immune system could be production of large amounts of antimicrobial peptides, a trade-off due to resource allocation. But the flies that were on a dietary feeding condition and the fully fed flies both showed this cost, so it seems unlikely that resource allocation alone explains the reduction in longevity. Alternatively, NFκB-related signaling could modulate the aging process directly or indirectly, through insulin signaling or stress response. Still another possibility is that immune-challenged flies accumulated molecular damage through microbial peptides or inappropriate apoptosis (Libert *et al*. 2006).

In a review by Schulenberg *et al.* (2004) the innate immunity of *Caenorhabditis elegans* was reviewed. The authors also looked at the trade-off between the immune system and host life history traits. They suggest that there is a switch that changes the resource allocation for the trade-off between immunity and other traits. This switch can mediate development and reproduction against immunity. It is required that this switch responds to developmental and environmental signals. The insulin-like receptor pathway is known to respond on environmental cues and stage-dependent neuronal signals. When the insulin-like receptor pathway is activated resources are used for development and reproduction and the expression of stress and pathogen resistance genes is suppressed. When the pathway is downregulated the expression of genes for development and reproduction is decreased and the expression of genes for stress response and pathogen resistance is increased (Schulenburg *et al*. 2004).

A study by Cotter *et al.* (2004) used the Egyptian cotton leafworm *Spodoptera littoralis* to examine the quantitative genetics of the innate immune function and other life history parameters by the use of a full-sib/half-sib design. In this study the immune system of the organism was not challenged. The authors looked at different trade-offs within the immune system by a restricted estimate maximum likelihood procedure. Hereby heritability of each trait and genetic correlations between traits can be estimated. A potential genetic trade-off was found as haemocyte density was positively correlated with cuticular melanisation and PO activity but negatively genetically correlated with antibacterial activity. This corresponds with the several studies that found evidence for a trade-off between the humoral and cellular parts of the immune system (Cotter *et al*. 2004).

There is still much unknown about the genetics underlying a trade-off. However some studies give important suggestions to research further.

Libert *et al.* (2006) suggest that the NFκB-related signaling could directly or undirectly play a role in a trade-off between the immune system and longevity. Indirectly, it could affect insulin signaling or stress response, which are aging-regulatory pathways. Schulenberg *et al.*

(2004) also suggests that the insulin-like receptor pathway could be the switch that controls the expression of certain genes playing a role in the trade-off between the immune system and other traits. Cotter *et al.* (2004) studied genetic correlations between the immune system and other life history parameters. The result that cuticular melanisation and PO activity is positively correlated with haemocyte density but negatively correlated with antimicrobial activity is important. Studies in this area should take into account that within the immune system trade-offs could also play a role. By studying the trade-offs between immunity and other traits results can differ between a cellular or humoral challenge.

Discussion

In this review, several mechanisms underlying trade-offs between the immune system and other traits have been described. The various studies described in this review varied in model organism, method to challenge the immune system, parameters to measure the correlated traits and many more aspects. This could explain that some studies found a mechanisms and other studies did not found a mechanism. Studies measured the costs of different aspects of the immune system; maintenance and deployment as described by Silva-Jothy *et al.* (2005), although sometimes these two could not be fully separated. For example, higher resistance *D. melanogaster* lines had twice the density of haemocytes as the control lines. To keep this level of haemocytes twice this high could be accounted as a maintenance cost, but when there is an immune challenge it becomes difficult to determine what you are going to measure as the deployment cost. This could be one reason why the suggested mechanisms underlying the various trade-offs differed.

The mechanisms suggested by the different studies can be linked to the knowledge of genetic studies on trade-offs, to try to deduce the genetic basis for the trade off. The most commonly used explanation for the trade-off between immunity and other traits is resource allocation. An organism has a restricted energy budget that it can spend on traits. When the immune system is challenged there remains less resources for the other traits to express themselves. Some studies suggested there is a switch that arranges the resources for the immune system and other traits (Kraaijeveld *et al*. 2000; Schulenburg *et al*. 2004). Two studies are suggesting that this switch could be the insulin-like receptor pathway (Schulenburg *et al*. 2004; Libert *et al*. 2006). The timing to turn the switch is of major importance. When the organism is in the developmental stage and the trade-off is already working this could lead to evolutionary costs while in the adult stage the costs can be recovered in the lifetime of the organism.

Recognition of pathogens stimulates the Toll and Imd pathways to induce a respons. However mainly Toll plays also a big role in the development the dorsal-ventral embryonic development in *Drosophila* (Wu & Anderson 1997). It seems that developmental signaling and immune signaling occur thru the same toll-pathways (Tauszig *et al.* 2000), it could be that this mediates trade-offs during the developmental stage.

An other mechanism in which a trade-off between the immune system and other traits operates is because the activation of the immune system is harmful in itself. Liberte *et al.* (2006) suggested that the production of antimicrobial peptides could cause molecular damage.

This review has shown that the mechanisms underlying a trade-off between the immune system and other traits found, frequently differ between studies. Because the immune system consists of many different aspects, it is likely that there are several different trade-offs. Comparing trade-offs between different organisms is tricky because organisms differ in many aspects. When looking at the link between mechanisms of trade-offs and the genetic basis, a

few studies already gained information for a better understanding of this genetic trade-off. However, much detail of the mechanisms remain unknown.

For future studying there are many opportunities to get a better understanding of trade-offs between the immune system and other traits.
The energy metabolism underlying the trade-offs of an organism is interesting. Some of the reviewed studies suggested that the amount of food; limiting food or *ad libitum*; is of great importance to see if a trade-off is occurring, the studies took food acquisition equal to the resources an organism has. However this is only partly true. Food gives an organism resources, but also the activity and rest of an organism are determining for the amount of resources that is left for the expression of traits. With a more complete view on the energy metabolism, there will be a more complete view on the expenditure of resources and hereby trade-offs can be better understood.
Some studies had a method by which they did not knew if the maintenance costs were measured or the deployment costs. It is important to distinguish these costs, if an organism has maintained its immune system very well and an immune challenged arrives, it could be that it has to use less deployments costs in comparison to an organism that did not maintained its immune system. If the costs are not well distinguished, the results of the study can be misinterpreted.
At last deducing the genetic basis underlying mechanisms of trade-offs between immunity and other traits is interesting. It could explain the variation of the expression of traits among individuals. It could also tell us more about eventual heritability aspects of trade-offs and hereby increase our knowledge of trade-offs on an evolutionary level. If dysfunctioning of the immune system is caused by a trade-off, the genetic basis underlying the mechanisms of trade-offs could be important for curing diseases.

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