Chapter 6

General discussion

Genomic instability in streptomycetes has been recognized for decades and has puzzled scientists since its discovery. Initial studies mainly focused on explaining the phenotypic traits it influences (*17*, *20*, *134–136*, *170*, *218*, *219*), its rate in different species (*17*, *20*, *134–136*, *218*), the reagents that affect it (*17*, *24*, *133*, *135*, *218*) and the patterns of chromosomal rearrangements (*20*, *33*, *134–136*, *170*, *211*, *219–221*). Despite this, little research has been done to understand the ecological and evolutionary function of genomic instability in *Streptomyces*. In this thesis, I examined the role of genomic instability by interpreting it as a division of labor, in which the population redirects the cost of antibiotics to a minor proportion of cells through terminal genomic rearrangements, thus maintaining the overall fitness of the colony while maintaining high levels of antibiotic production. Furthermore, I investigated the fate of these altruistic mutant cells after their emergence and find that there is a process analogous to a mutational melt-down occurring through the accumulation of both point mutations and large genomic deletions. This leads to a rapid reduction of fitness in mutants and provides the potential to flexibly adjust caste ratios in reaction to environmental changes. To understand the molecular mechanisms that cause the trade-off between fitness and antibiotic production, I used a proteomics approach to identify both global and specific changes in protein abundance underlying the altered fitness and antibiotic secretion in response to genomic rearrangements (Fig. 1).

Division of labor in *Streptomyces*

Like in human society (*222*), division of labor (DoL) is important for the coordination of complex biological systems. In microorganisms, DoL allows specialized tasks to be carried out more efficiently by collaboration and coordination between different cell types (*49*, *50*). For example, *Bacillus subtilis* promotes migration through the labor divided between surfactin-producing and matrix-producing cells. Some cells produce surfactin to reduce friction, which allows matrix-producing cells to form Van Gogh Bundles to migrate (*121*). During infection, pathogenic yeast, *Cryptococcus gattii* divides a subpopulation of cells to produce tubular mitochondria in response to host reactive oxygen species. This subpopulation can protect the rest of the cells in macrophages in helping them to proliferate intracellularly (*223*). These examples from species in various taxa suggest that DoL is a vital strategy in the evolution of microorganisms, which may have been instrumental in the evolution of multicellular life.

Multicellularity in *Streptomyces* has been considered as a canonical example of DoL given that these bacteria form germ cells (aerial mycelia and spores) and somatic cells (vegetative mycelia) (*51*, *59*). Dispersed spores germinate and form vegetative mycelia that forage for nutrients to support growth. Later on, when nutrients are depleted, aerial mycelia will form and produce chains of spores that eventually give rise to another cycle of life (*7*, *8*). During this transition, secondary metabolites including antibiotics are produced by

vegetative mycelia for the benefit of the whole colony (*10*). In addition to this example of DoL, our results in **Chapter 3** highlight a new type of DoL for antibiotic production. In our scenario of DoL, a proportion of cells in a colony undergoes genomic rearrangements that terminally differentiate into a "sterile caste" specializing in diverse antibiotic production at the cost of fitness. Overall, the colony benefits from the sacrifice of this proportion of "sterilized" cells and gains advantages in competition with other bacterial species. This is comparable to classical DoL between sterile helpers and reproductive castes in social insects (*123*). Because mutant helpers in *Streptomyces* are derived from the WT through genomic rearrangements, the genetic information in mutants is also present in WT, meaning the fitness interests between these two types of cells are well aligned. Following Hamilton's rule (*224–226*), this high relatedness would allow the maintenance of altruistic traits in mutants, thus leading to the DoL in antibiotic production.

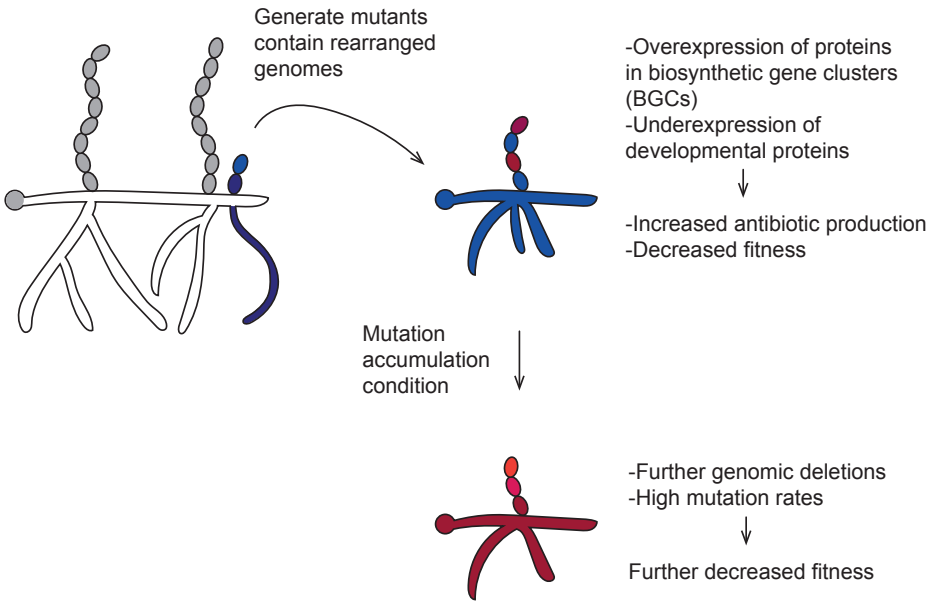

Fig. 1. Schematic summary of this thesis. *Streptomyces* generates mutants containing genomic rearrangements at a high frequency. Mutants produce increased antibiotics at a cost of reduced fitness due to the expression changes in relevant proteins. Under the mutation accumulation condition where natural selection is minimized, mutant cells will continuously accumulate genomic deletions leading to further decreased fitness. Gray colored spores and hyphae contain the intact wild-type genome. Blue and red colored spores and hyphae contain the genome with various degrees of rearrangements.

Phenotypic variation, as a prerequisite for DoL, can be achieved at both genetic and nongenetic levels (*227*). Among many DoL examples in a single species, nongenetic variation is frequently used as a mechanism to create phenotypic variation (*50*). However genetic variation including changes in DNA sequences and epigenetic inheritance can also happen in a population derived from isogenic parents (*58*, *228*, *229*). In known microbial DoL examples, it appears that most genetic changes in DNA sequences are attributable to point mutations. It is however not common to observe phenotypic variation generated by massive genomic rearrangements as in *Streptomyces*. Our finding is specifically compelling from this perspective, because it has been argued that phenotypic variation caused by genetic changes would not be favored in most conditions due to a high chance of being invaded by cheaters caused by reduced relatedness. I believe the DoL shown in **Chapter 3** is favored because of two relevant reasons. Firstly, the mutant genomes are highly related to the wild-type genome to support kin selection. Secondly, because the filamentous nature of *Streptomyces* makes cells physically connected to each other, it also makes invasion of cheaters difficult to occur. Evidence of DoL in filamentous cyanobacteria and cable bacteria where cells are also physically connected suggests this might play an important role in maintaining a stable DoL (*62*, *174*). Further studies in testing whether DoL is more widespread in physically connected bacteria will help to understand the role of the second factor.

Another interesting aspect of this type of DoL concerns whether the genomic rearrangements are a cause or a consequence of the emergence of altruistic helpers. I hypothesize that the emergence of mutant cells within the mycelium is a stochastic process: during colony development, some cells are prone to genomic damage, leading to mutants that consequently produce antibiotics rather than reproduce. Similar outcomes are observed in some green algae, such as *Volvox carteri* in which cell sizes correlate with the tasks they perform for the colony: cells larger than 8 μm specialize at reproduction and growth while smaller cells specialize at locomotion, constructing a classical germ-soma DoL (*230*, *231*). These smaller somatic cells, interestingly, undergo programmed death which is comparable to altruistic mutants in *Streptomyces* (*232*, *233*). Studies have shown that larger germ cells and smaller somatic cells are more efficient at performing their local tasks (*234*), respectively, supporting the idea that fitness return in various cell types is a key factor in determining the tasks they perform. However, cell differentiation seems to be regulated and programmed in volvocine algae. Future studies on elaborating our hypothesis about *Streptomyces* DoL will benefit from comparing it to research in *Volvox*. Many follow-up questions can be asked: is this type of DoL genomically encoded? Is it an evolutionarily stable strategy? Can conditions alter the ratio between altruistic mutant helpers and WT reproducers as in some other species? These require more research in the future and are fundamentally important for both the *Streptomyces* and microbial evolution fields.

I have demonstrated that producing antibiotics is a costly activity that dramatically trades off with fitness, meaning that cooperative cell types are likely favored to exist. An extreme example of this type of mutually incompatible tasks would be nitrogen fixation and photosynthesis in some cyanobacteria (*99*). Our findings help to understand the ecological functions of antibiotics produced by *Streptomyces*, which possibly explain why cryptic antibiotics, that are predicted in genome but not produced in lab conditions, exist in many *Streptomyces* species (*145*, *235*). From an application perspective, knowing that producing antibiotics is costly also raises a new idea about how to find new antibiotics from "old" *Streptomyces*. Future studies focusing on testing whether similar DoL are present in other *Streptomyces* would be helpful to prove if DoL in antibiotic production mediated by genomic rearrangements is a common rule that can be applied in many *Streptomyces*. And if so, then it could possibility lead to novel solutions to elicit new antibiotics.

Mutational meltdown due to massive genomic deletions

From a mechanistic perspective, it is impressive to observe that overproduction of antibiotics is directly linked to genomic rearrangements, specifically large deletions located at the ends of the genome. Older studies have focused on elaborating the pattern of genomic changes rather than focusing on their impacts on cells. In **Chapter 4**, I studied how large genomic deletions influence the evolution of these sterile mutants and what their fate will be over time. In our experimental set-up, we simulated the spore-to-spore transfer which maximizes genetic drift while natural selection is minimized. This is very similar to a mutation accumulation experiment which also allows us to estimate the mutation rates of different lineages.

In our experimental condition, cells were transferred through single cell bottlenecks, leading to a small population size. Considering that *Streptomyces* propagate asexually, this allows us to see the effect of Muller's ratchet, a process in which deleterious mutations accumulate irreversibly in a population lacking recombination (*179*, *180*). This will further lead to mutation accumulation in all lineages. We observed the meltdown in mutant lineages but not WT ones over a period of 25 transfers. First, mutants had a higher base-substitution mutation rate and smaller effective population size, explaining why a mutational meltdown (*182*) is likely to happen more rapidly in mutants compared to WT lineages. Second, lineages with terminal deletions quickly accumulate further deletions which in turn decrease the fitness and thus reduce the effective population size. These two effects together cause mutants in *S. coelicolor* to undergo an irreversible and accelerating mutational meltdown (Fig. 2). This fits the idea of a classical mutational meltdown, in which a small population going through Muller's ratchet experience accelerating fitness declines caused by deleterious mutations (*182*).

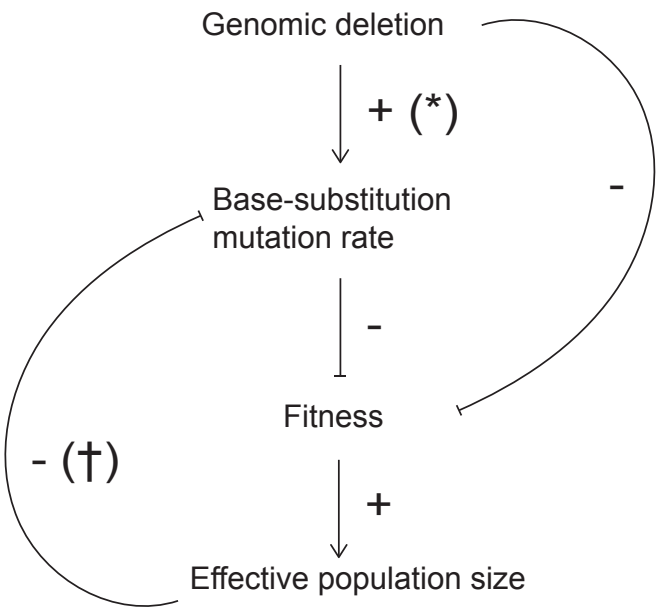

Fig. 2. Mutational meltdown in *Streptomyces coelicolor*. Genomic deletions result in a higher base-substitution mutation rate and lower fitness. The latter subsequently leads to a smaller effective population size. According to Drake's rule (*) (*236*) and drift-barrier hypothesis (†) (*237–239*) respectively, these genomic deletions and the smaller effective population size will lead to a higher base-substitution mutation rate. Taken together, these summarize a mutational meltdown in *S. coelicolor*.

Proteomic level changes due to genomic rearrangements

Chapter 5 demonstrates that genomic rearrangements increase the expression of proteins from antibiotic biosynthetic gene clusters (BGCs) and decrease the expression of many developmental proteins, which is consistent with the trade-off we observed in our studies. The fact that different sized genomic rearrangements result in similar proteomic changes in these proteins is intriguing, because it brings up new questions about how large genomic rearrangements affect protein expression. Future studies on finding the quantitative connections between omics data by using mutants with strictly controlled levels of genomic rearrangements will be important in elaborating the genetic network underlying phenotypic changes to spore and antibiotic production. This may be challenging because classical molecular studies in explaining gene networks requires precise knock-out strains. However, as we have observed that mutants with diverse genomic rearrangements universally behave similarly, studies using omics approaches can provide a good beginning in providing directions for further detailed studies.

Genomic instability and adaptation

Genomic instability exists in a wide range of *Streptomyces* species. For the first time, this thesis explains what the evolutionary function of genomic instability is by linking it to a DoL. However this does not exclude the possibility that genomic instability can play other roles in the evolution of *Streptomyces*. For instance, some filamentous actinomycetes have the ability to extrude specialized cells in hyperosmotic conditions that frequently harbor rearranged chromosomes, suggesting genomic instability might be a way of rapidly adapting to certain stressful environments (*240*). Another study where *Streptomyces clavuligerus* was evolved by competing it serially against methicillin-resistant *Staphylococcus aureus* resulted in strains overproducing the antibiotic holomycin, caused by deletion of a large megaplasmid (*241*). These studies suggest that genomic instability is an important strategy for *Streptomyces* to adapt to changing environments. Experimental evolution will be a powerful approach to test relevant hypotheses. For example, we can set up a condition where *Streptomyces* competes with another bacterium and test frequencies of evolving lineages harboring genomic rearrangements. Reciprocally, we can test whether a *Streptomyces* strain with a higher level of genomic instability will evolve to produce antibiotics more rapidly. Similar studies have been done in testing functions of bacterial mutators in gaining antibiotic resistance, in which mutators readily become antibiotic resistant while selecting resistant strains also result in mutators. Comparing genomic rearrangements to point mutations will be important, because both of them are double edged swords for bacteria themselves due to the deleterious effects of many mutations. Identifying the advantages of these two types of mutations will help us to understand their evolutionary importance.

As discoveries in modern science requires interdisciplinary knowledge, fundamental research in microbiology requires a comprehensive understanding in both evolutionary and molecular biology. I have used a breadth of techniques to study fundamental evolutionary questions in this thesis. Overall, this research will benefit both evolutionary biologists and molecular microbiologists with the blossoming prospect of the discovery of new antimicrobials that are so urgently needed.