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The prospects of automation in drug discovery research using silkworms

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SUMMARY We have established several models of infectious diseases in silkworms to explore disease-causing mechanisms and identify new antimicrobial substances. These models involve injecting laboratory-cultured pathogens into silkworms and monitoring their survival over a period of days. The use of silkworms is advantageous because they are cost-effective and raise fewer ethical concerns than mammalian subjects, allowing for larger experimental group sizes. To capitalize on these benefits, there is a growing importance in mechanizing and automating the experimental processes that currently require manual labor. This paper discusses the future of laboratory automation, specifically through the mechanization and automation of silkworm-based experimental procedures.

Keywords silkworm, experimental infection, alternative animals, laboratory automation

1. Importance of alternative experimental models

Modern medicine and health science have significantly contributed to our well-being, owing much to the invaluable sacrifices of laboratory animals. Research conducted with mammals, especially mice, has been pivotal in advancing from our fundamental science to practical clinical applications. Nonetheless, increasing animal welfare awareness has made it difficult to conduct experiments using mammals in the field of research and development in non-medical fields such as food and cosmetics.

In the pursuit of alternatives to using mammals in research, the concept of employing silkworms in health science has been explored. This idea has been put forward by various research teams, including ours, for approximately two decades (*1-17*). Such studies have shown that *Staphylococcus aureus* infects and kills silkworms and that the therapeutic efficacy of antibiotics can be evaluated in the same model, and have searched for compounds that are effective in treating infections from soil bacteria libraries. As a result, a new antibiotic, Lysocin E, was identified using the silkworm assays, and was also found to be therapeutic in mice. Traditionally, life science research involving animal testing has relied on mammals, like mice. The ethical dilemmas and the high costs associated with mouse experiments present considerable challenges in this field of research. We propose that these problems can be overcome by using invertebrate alternatives, such as the silkworm (*5,10,14,16-22*).

Silkworms facilitate easy administration of precise sample doses, enabling detailed evaluation of drug effects on an individual basis. Additionally, the low cost of silkworm-based experiments presents a stark contrast to the expenses incurred with mouse models. A typical research laboratory in an academic or government setting is capable of assessing up to 100 chemical compounds daily with silkworms, using a standard dose for three trials per compound, totaling the use of 300 silkworms.

2. Limitations of silkworm models

While the experimental system using silkworms has the above advantages, there are also technical challenges to be overcome before it can be more widely used as a research and development platform in the future. For example, there is a lack of standardization of the method to rear the silkworms in university laboratories and other environments (Figure 1). Indeed, some of our collaborator groups sometimes experience problems with silkworm rearing (the process of hatching the eggs to make them into 5-instar larvae). There is a lack of documentation that summarizes a unified view on what specifications of equipment are required to rear silkworms stably using facilities such as universities (*e.g.*, volume of incubators, types of containers used for rearing silkworms). Furthermore, in infection experiments using silkworms,

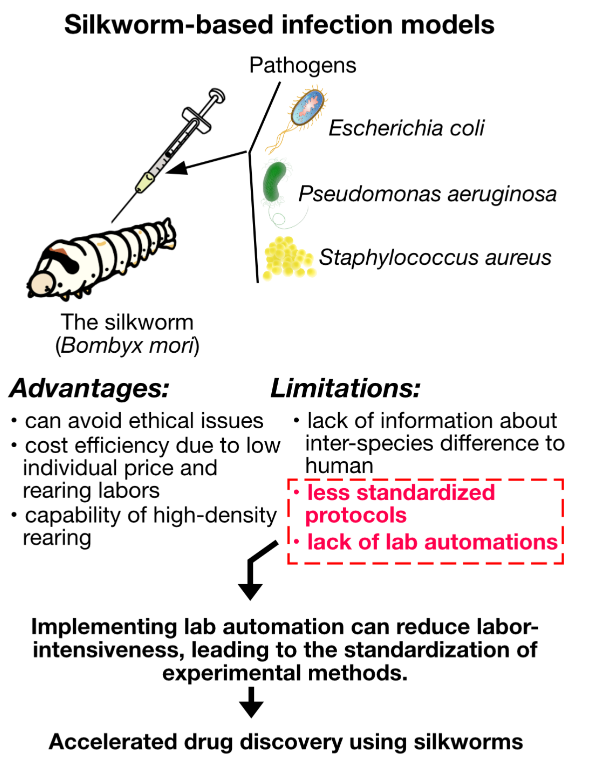

Figure 1. Prospects of silkworm-based infection models. By implementing lab automation, we can reduce labor-intensiveness, leading to the standardization of experimental methods. This will accelerate drug discovery research using silkworms.

a syringe with a needle for tuberculin (typically 1 mL) is used to inoculate a sample such as a bacterial solution directly into the hemolymph. Not only does learning this technique require a relatively long period of training, but there are also risks such as needlestick accidents. Due to the nature of the silkworm-based experimental system, a large number of individuals (several hundred or more) are used in a single experimentation, resulting in frequent injection operations and a high risk of needlestick accidents. The authors' laboratory currently rears more than 6,000-10,000 silkworms for experiments every month (*i.e.*, on the scale of 100,000 silkworms per year), so if the probability of a needlestick accident is about 1 in 100,000 (*i.e.*, very rare), this means that about one needlestick accident would occur every year. So far, no severe accidents have occurred, but this is a serious issue from the perspective of promoting the wider use of experimental systems using silkworms in the future.

The observation of silkworms after inoculation with the microorganisms (monitoring survival numbers) is also a major burden for the experimenter. With 240 silkworms at a time and a sample size of 4 silkworms per group, this gives an experimental design of 60 groups. In addition, to date, no criteria have been established for determining whether a silkworm infected with pathogenic bacteria is alive or dead, which has led to variations in judgement between individuals. Therefore, when observing a large number of silkworms several times a day, errors or misses may occur in the silkworm observation records. Furthermore, if observation is dependent on human labor, it is impossible to conduct observations at nights or at other times when the experimenter is not present (*e.g.* Sundays). Therefore, issues also remain in terms of the comprehensiveness of the observation records.

3. Perspectives of laboratory automation

Overcoming the above-mentioned challenges necessitates a push towards maximizing the mechanization and automation of silkworm-based experiments. Beyond automating the rearing and injection processes, leveraging the rapidly advancing technologies in object recognition and detection can facilitate the automation of the observation process as well.

Efforts are underway to automate various processes in insect research, including studies on silkworms. This includes advancements in the automation of rearing, experimental procedures, and result monitoring. There's a growing trend in utilizing automated systems for insect rearing to improve the efficiency of producing populations for experiments. With the aid of image processing and embedded systems, it is now possible to continuously monitor and adjust the temperature and humidity levels crucial for the growth of silkworm larvae. Moreover, with the application of deep learning, systems are being crafted that can accurately differentiate between healthy and unhealthy silkworms, which is further streamlining and automating tasks within the sericulture industry (*23*).

In addition, there are many manual processes involved in measuring nematode longevity, and the separation of adults and larvae for experiments has been done manually. However, Felker, *et al*. reported the automation of this process (*24*). Automation has also been implemented in the injection of samples into insects and nematodes. Cornell *et al*. created a system that automatically injects nucleic acids (DNA and RNA) and drugs into *Drosophila* embryos aligned on sheets for transgenic operations. Embryos aligned by the novel device are imaged and recognized by the software, and samples are automatically injected (*25*).

By using specialized camera technology on nematodes, the system can automatically recognize and locate the nematode, determine the success of the injection, and microinject the sample into the nematode at a rate of one every 10 seconds (*26*). The use of automated systems for monitoring insect behavior and ecology is also being studied. Insect monitoring of wing-beat harmonics, melanization and direction of flight are conducted by sensing near-infrared light scattered from behind the insect (*27*).

In addition, the use of smartphones to automatically quantify the number of *Drosophila* offspring and the measurement of adult parameters is being attempted

(*28*). Xu *et al*. used a mobile phone and a macro lens to measure the body length and head width of the larvae of the fall armyworm, and analysed and collected these data using the random forest technique, which resulted in a 92.22% accuracy in identifying the number of larval instars, ranging from 1 to 6 instars. (*29*).

Furthermore, recently, it has become easier for personal computers to perform various computational processes based on images acquired from cameras and other sources by using open-source image recognition toolkits such as OpenCV. By utilizing machine learning libraries such as PyTorch, which runs on the Python programming language, based on the obtained image information, it is expected to be possible to recognize silkworms in real time and obtain numerical information on their appearance and behavior, thereby providing a development platform for the automation of survival count observation.

4. Concluding remark

By advancing mechanization and automation efforts, a framework can be developed that allows researchers to carry out only the essential tasks in the lab, enabling them to automatically gather experimental data without being physically present. Our research team is focused on pioneering the automation of silkworm experimental processes to facilitate an environment where researchers can devote more time to intellectual tasks by minimizing the labor and time spent on manual lab work. Automating the processes involved in rearing, experimenting, and observing is anticipated to become a critical component for broadening the application of silkworm-based research platforms in various fields.

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