The Canadian Entomologist (2024), **156**, e40, 1–14
doi:10.4039/tce.2024.36

Published on behalf of the Entomological Society of Canada

ARTICLE

Automatic counting and identification of two *Drosophila melanogaster* (Diptera: Drosophilidae) morphs with image-recognition artificial intelligence

Aaron Gálvez Salido, Roberto de la Herrán, Francisca Robles, Carmelo Ruiz Rejón, and Rafael Navajas-Pérez

Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Granada, Spain
Corresponding author: Rafael Navajas-Pérez; Email: rnavajas@ugr.es

(Received 28 May 2024; accepted 20 September 2024)

Abstract

Many population biology, ecology, and evolution experiments rely on the accuracy of the classification of individuals and the estimation of size population. The visual classification of vinegar flies, *Drosophila melanogaster* (Diptera: Drosophilidae), morphs is a laborious task usually performed by bench workers. Because of the size of the flies and the degree of precision needed to distinguish the morphological features on which the classification is based, the work is performed using a dissecting microscope. Here, we describe a method to automate the counting and identification of two types of vinegar flies, white and wild individuals. Our method is based on the image-recognition artificial intelligence (AI) tool, FlydAI (FlyDetector AI), which proved to correctly classify the flies when high-quality images were used, with a success rate of up to 100% in samples containing up to 200 individuals. This is a significant improvement with respect to preexisting approaches in terms of accuracy and specificity of the morphs detected. Although this tool is exclusively trained to routine lab tasks involving wild and white *D. melanogaster*, the AI can be easily trained to recognise different vinegar fly mutants and other types of insects of similar size, and its potential in other areas still needs to be explored.

Introduction

Around 4000 labs worldwide work on *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae), the vinegar fly; according to the Bloomington *Drosophila* Stock Center, BDSC, the database of which includes more than 3900 research groups in 72 countries. Although the first documented experimental use of *Drosophila* was by William Castle's group at Harvard in 1901, this species became a model insect after Thomas H. Morgan and collaborators laid the foundations of the field of genetics in their famous Fly Room at the beginning of the twentieth century (Morgan 1909). Since then, hundreds of mutations have been described in *D. melanogaster* causing alterations in wings, abnormal body colour, odd-coloured eyes, and strangely formed heads (Chadov *et al.* 2015). These mutations allow a direct association between phenotypes and genotypes, making them easily scored markers for genetic mapping, population biology, ecology, and evolution studies (Kohler 1994). *Drosophila melanogaster* enabled seminal genetics discoveries, such as the nature of Mendelian factors, physical mapping, and the effect of X-rays in chromosomal structure (Morgan 1909; Jennings 2011), and was used to test the genome

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sequencing shot-gun method 11 months before the publication of the human genome (Kohler 1994; Adams *et al.* 2000; Rubin and Lewis 2000).

The vinegar fly is also the subject of a large number of experiments in a wide range of different fields (Hales *et al.* 2015). It is a key model for regenerative biology and medicine. Well-established protocols exist for its genetic modification, and many orthologous genes are available to study mechanisms underlying human disease (Yamamoto *et al.* 2014), including cancer, cardiovascular disease, and neurological diseases (Bellen *et al.* 2010; Pandey and Nichols 2011). *Drosophila melanogaster* is also considered a model organism for developmental biology. Over the past four decades, *Drosophila* has become a predominant model to understand how genes direct the development of an embryo (Jennings 2011). Many driver lines from *Drosophila* are used to provide spatiotemporally regulated genetic handles to almost every cell type (Chan *et al.* 2024). The use of vinegar flies is also common in studies related to the influence of genetic and environmental factors in behaviour, such as mating and courtship (Balaban-Feld and Valone 2018), aggression (Baier *et al.* 2002), and learning and memory (Maggu *et al.* 2022).

For some analyses, only a simple count of total number of individuals is needed, whereas for others, detailed counts (*i.e.*, classifying different morphs) are required. Importantly, the efficient estimation of the number and type of individuals is crucial in experiments that correlate certain phenotypes with specific genotypes, as is the case for genetic mapping (Zhai *et al.* 2003) or in functional ecology studies (Mendes *et al.* 2021). In other cases, a measure of the raw number of individuals in a population is required, such as in fecundity experiments (Nouhaud *et al.* 2018). The traditional manual process includes a short treatment of the flies with anaesthesia, after which they are counted and identified by experienced technicians using dissecting microscopes. In some other cases, it is important to track fly movement, as in some behavioural experiments (Nichols *et al.* 2012).

Although the short lifecycle of *Drosophila* enables the possibility of performing experiments with large numbers of individuals, some methodological bottlenecks remain. The reduced size of the individuals (about 3 mm long) and their motility often hinder their management and identification and make it difficult to track movement or to make observations of certain behaviours (Macartney *et al.* 2022). Counting large populations of flies is a tedious and time-consuming process. The total processing time varies and depends on the ability of a human operator and the number of individuals required. The typology of some studies requires analyses of high numbers of individuals. In these cases, automated phenotyping is a possible solution. These methods use deep-learning models to automatically recognise and categorise elements within images and consist of several steps: (1) data collection, typically a digital image; (2) identification and classification of the objects in the image by the artificial neural networks; (3) image pattern recognition, where a predefined class label is assigned to an image, or part of an image, and tagging using object bounding boxes; and (4) segmentation, which is necessary for counting and recognising individual objects (Uchida 2013). In *Drosophila*, examples include (1) methods to efficiently count large number of individuals and (2) methods able to determine the offspring size and gender ratio in fly populations. All of the methods use as inputs images of anaesthetised flies or images of flies in sticky traps. Within the first category of examples, the most significant tools are FlyCounter, a program coded in MATLAB (Yati and Dey 2011), and an application for Android devices that is an adaptation of a preexisting app to count seeds (Karpova *et al.* 2020). Other authors, using ImageJ (Nouhaud *et al.* 2018; Ng'oma *et al.* 2020) and proprietary software (Waithe *et al.* 2015), have developed methods to indirectly make estimations of *Drosophila* population sizes based on the number of eggs laid. Within the second category of examples, there is an application for cell phones, also named FlyCounter (Genaev *et al.* 2022), that is based on a YOLOv4-tiny neural network algorithm and an image-based object detection method that uses deep convolutional neural networks to identify *D. suzukii* individuals (Roosjen *et al.* 2020). Aside from counting the number of flies, the latter two approaches can also

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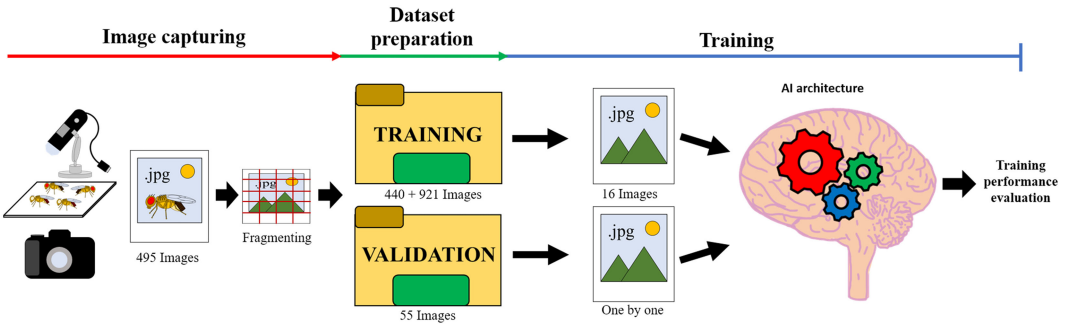

Figure 1. Pipeline of the development and use of image-recognition AI: image capturing, dataset preparation, and AI training and validation.

differentiate individual flies according to their sex. None of the methods developed to date has been trained to differentiate *Drosophila* mutants.

Here, we present FlyDetector artificial intelligence (FlydAI), an image-recognition algorithm based on TensorFlow, that can be used to count and classify large populations of wild-type and white-mutant *D. melanogaster*. The FlydAI uses images containing different numbers of flies and makes predictions on the total number and morphs of flies.

Materials and methods

The rationale behind the present experiment was to capture images containing two types of *D. melanogaster* flies so that they subsequently can be processed using *ad hoc*–trained image-recognition AI that automates the counting of individuals and the identification of the different phenotypes. A process map is shown in Fig. 1.

The workflow of FlydAI includes (1) an image in joint photographic experts group (jpeg) format is taken as input; (2) images exceeding 1094 × 768 pixels are cropped into fragments; (3) each fragment is processed individually, individuals are identified, classified by phenotype, and recorded; (4) a summary of the numbers of individuals and phenotypes counted is generated as a comma-separated values (csv) file; and (5) the original image is reconstructed using the interpreted fragments (Fig. 2).

Image dataset preparation

Wild-type and white-mutant *D. melanogaster* flies were anaesthetised with ether for 45 seconds, placed onto blue, white, pink, yellow, brown, and gridded cards (see Supplementary material 1) to maximise background subtraction (Uchida 2013), and photographed. The number of individuals per image ranged randomly from 1 to 10. For replicates, individuals were randomly replaced and repositioned to train the model to identify different flies in different positions. A total of 350 flies were used. Images were captured with two different devices: a Canon EOS 70D camera with a 100mm f/2.8L macro lens (Canon, Ota City, Tokyo, Japan) and a Bysameyee 8-SA-00 digital microscope (Bysameyee, South Korea). A dataset of 495 images in three categories (only white mutants, only wild-type flies, and both white mutants and wild-type flies) was generated.

Development of AI for image recognition

FlydAI training was performed using TensorFlow, a free and open-source software library for machine learning and artificial intelligence (https://www.tensorflow.org). After conducting an

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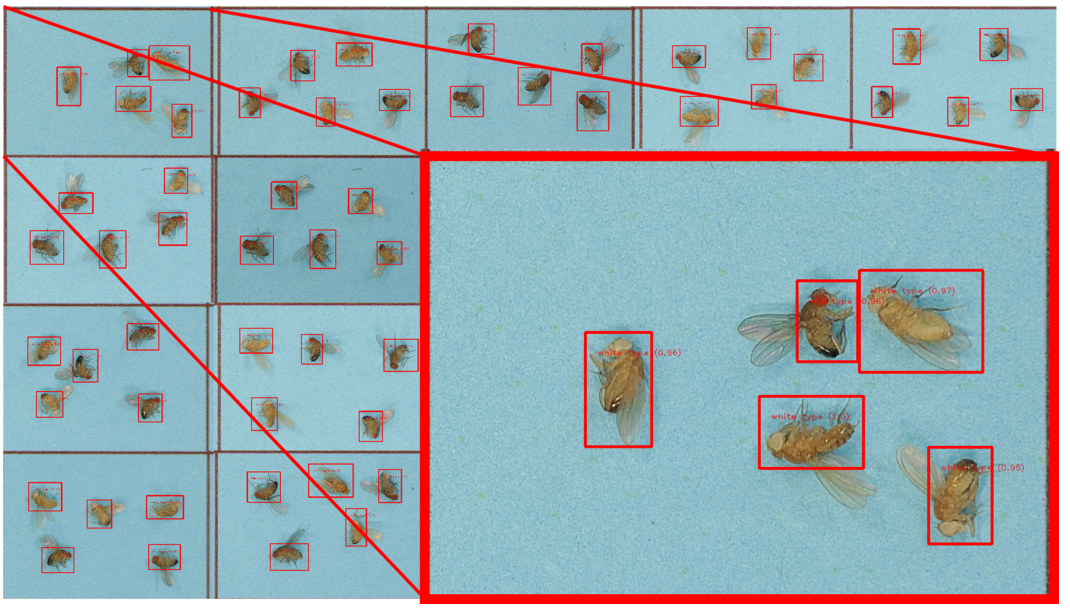

Figure 2. Output of an image reconstructed by FlydAI. Each individual is identified by a red square; phenotypes and a value of confidence (a cutoff value of 0.65 was considered) included in brackets are indicated inside.

average accuracy (mAP) test with COCO (Common Objects in Context, https://cocodataset.org/; Lin *et al.* 2014) for five different architectures (for details, see Data availability), EfficientDet-Lite2 architecture (batch size = 16 for training and 1 for validation; number of epochs = 120) was selected because of its more efficient performance in terms of training and inference times.

The size of the images was standardised, so that images exceeding 1094 × 768 pixels were cropped into smaller fragments to meet this criterion. This facilitated the detection of small targets and was the optimal image size with which TensorFlow architecture was trained. Reducing or rescaling the image to suit the optimal size is not an option because doing so would reduce the level of detail and information of the image, thereby dramatically decreasing the model's performance. When cropping images, flies that are positioned on a boundary between two fragments may be misclassified or classified twice. To avoid these potential problems, we recommend using a grid card (see Supplementary material 1) to place the anaesthetised individual flies well within the squares.

Wild-type and white-mutant flies were manually identified in the images and labelled with labelImg (https://github.com/tzutalin/labelImg). Images were randomly distributed for training (440; 89%) and validation (55; 11%). For training, we aimed to increase the variability of the dataset to help improve the AI's resistance to photographic artefacts. An additional set of images therefore was generated with Roboflow (https://roboflow.com; Fig. 3), using as the source the initial 440 images. The Roboflow software reconstructed 921 new pictures from the original images by randomly combining their different parts, including specular images, and by adding 5% artificial noise. As a result, a final dataset of 1361 images (440 original images + 921 Roboflow-created images) was obtained and used for AI training. Reports for each validation were generated and manually inspected.

Only the images that were neither labelled nor previously processed by the AI were used to test the AI. Sets of images with different fly densities, containing 25, 50, 100, and 200 wild-type and white-mutant flies in different percentages, were reconstructed from the dataset. For that task, different combinations of fragments containing up to 10 individuals were used. In addition, three

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Figure 3. Example of an image reconstructed by combining fragments and adding artificial noise generated by Roboflow.

sets of images used for testing were altered by adding 5, 15, and 30% noise with Photoshop (Adobe, Inc., San Jose, California, United States of America; an example is shown in Supplementary material 2) and then were used to test the AI as previously described. The whole process, using different sets of images with the same densities (25, 50, 100, and 200) and the same noise levels (0%, 5%, 15%, and 30%), was repeated 15 times. For each replicate, the combination of fragments differed. The run time (for Google Colab (Mountain View, California, United States of America) GPU: NVIDIA Tesla K80 (Austin, Texas, United States of America) was recorded.

Data availability

A GitHub repository was created to allocate the Python scripts written to automate the process, the compiled programs, a step-by-step protocol, and the dataset used in this study: https://aarongs1999.github.io/Drosophila_AI_Tensorflow/Drosophila_AI_Tensorflow.html. Users can reproduce the analysis presented here using the sample dataset provided, run the model using private images of white and wild *Drosophila*, or train the model using images with different materials.

Human determination of number and phenotype of individuals

Four experienced geneticists were asked to estimate the number of wild-type and white-mutant flies in digital images of different densities (25, 50, 100, and 200 flies). Each geneticist was given 15 images that were randomly selected from the dataset of high-quality images containing a mix of wild and white flies. The geneticists did not see the images before the experiment and did not know the number of individuals in each image in advance. The time to process each image – the amount of time from when the digital image file in question was opened until the count was finished – was recorded.

In addition, three experienced geneticists were asked to estimate the number of wild and white flies in lab samples of different densities (approximately 25, 50, 100, and 200 flies) using dissecting microscopes. Each geneticist was given 10 samples that contained a mix of wild and white flies. The geneticists did not know the number of individual flies in each sample in advance. The time

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needed to process each sample – the amount of time from when the sample in question was provided until the count was finished – was recorded.

Statistical processing of data

For each image, the success rate of the estimations of three parameters – total number of individuals, number of wild individuals, and number of white individuals – was calculated as follows:

$$\text{Success rate} = [(\text{experimental value}/\text{real value}) \times 100]$$

Values greater than 100 indicate overestimation, and those less than 100 indicate underestimation.

Results

FlydAI: individual counts, phenotype identification, and processing time

Individual counts. When images with a degree of noise of up to 5% were input, in all tests performed (densities 25, 50, 100, and 200) and for all 15 replicates, FlydAI estimated the correct number of individuals with a success rate over 99.8%. For images with 15% noise, the efficiency was better than 96%. In all of these cases, some flies remained not detected because the individuals were out of focus or in positions that hampered their identification, even by a human operator. In pictures with 30% artificial noise, FlydAI accurately estimated the number of individuals with an efficiency of 75.2%. Across the entire dataset, the average percentage of successful estimates of numbers of individual flies by the AI was 92.92% (Table 1).

Phenotype identification. Regarding phenotype identification, for images taken under controlled conditions of focus and light, all individuals previously detected by the AI were assigned a correct phenotype (wild or white) for all densities and in all replicates (Table 1). When images with 5% artificial noise were used as the input, all individuals were assigned the correct phenotype, except for one single wild fly that was phenotyped by the AI as a white fly. For images with 15% artificial noise, 82.3% of white individuals were assigned the correct phenotype. Wild individuals were overestimated by 15.6%, with some white individuals incorrectly assigned to the wild phenotype. For images with 30% artificial noise, wild individuals were overestimated by 47.74%, with most white individuals incorrectly assigned to the wild phenotype. In fact, using these images as the source, FlydAI correctly phenotyped only 3.67% of white-mutant flies (Table 1).

Processing time. The overall mean average run time for FlydAI was 75.83 seconds per image and did not differ significantly between the different replicates, regardless of the fly density or noise level (Table 1). The execution time was directly related to the complexity of the TensorFlow architecture. The architecture selected for the present study (EfficientDet-Lite2) proved to be more efficient of the options tested.

Human workers: individual counts, phenotype identification, and processing time

Individual counts. Human workers were asked to identify the number of white and wild individuals from both digital images and lab samples of different densities (25, 50, 100, and 200). For digital images, the average percentage of successful estimations of individual fly numbers overall was almost 100% (Table 2). For lab samples, the average percentage of successful estimations of individual fly numbers overall was 99.6% (Table 3).

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Table 1. Success rate (SR) of estimation of number and phenotype of individuals by AI and run time for the different densities and image sources

Fly density	SR: count	SR: ident. wild	SR: ident. white	Run time (seconds)
		Noise 0%		
D25	100	100	100	75,27
D50	99.87	100	100	79,6
D100	100	100	100	80,25
D200	99.7	100	100	80,14
Average	99.89	100	100	78,81
		Noise 5%		
D25	99.73	99.55	100.67	71.24
D50	99.87	100	100	77.01
D100	100	100	100	76.02
D200	99.77	100	100	75.28
Average	99.84	99.89	100.17	74.89
		Noise 15%		
D25	95.2	122.8	71.41	74.69
D50	94.53	128.3	68.07	74.29
D100	98.87	108.2	91.95	74.26
D200	98.47	101.46	97.5	75.49
Average	96.77	115.19	82.23	74.68
		Noise 30%		
D25	76.53	138.82	13.97	75.07
D50	61.73	130.04	0.51	74.56
D100	83.87	184.64	0	75.22
D200	78.63	137.47	0.19	74.96
Average	75.19	147.74	3.67	74.95
		Overall		
D25	92.86	115.29	71.51	74.07
D50	89	114.59	67.15	76.36
D100	95.68	123.21	72.99	76.44
D200	94.14	109.73	74.42	76.47
Average	92.92	115.7	71.52	75.83

Phenotype identification. The average percentages of correct phenotype identifications in digital images and lab samples were equal, both being 99.6%.

Processing time. For both individual counts and phenotype identifications, the mean average time required to complete each task increased with the density of flies. For digital images, the mean average run time was 51.13, 67.45, 84.14, and 153.09 seconds per image for densities of 25,

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Table 2. Success rate (SR) of estimation of number and phenotype of individuals from digital images by human workers and processing time

Fly density	SR: count	SR: ident. wild	SR: ident. white	Run time (seconds)
D25	100.36	99.34	101.61	51.13
D50	100.18	100	100.46	67.45
D100	100.07	100.6	95.59	84.14
D200	99.54	98.78	100.67	153.09
Average	100.04	99.68	99.58	88.95

Table 3. Success rate (SR) of estimation of number and phenotype of individuals from lab samples by human workers and processing time

Fly density	SR: count	SR: ident. wild	SR: ident. white	Run time (seconds)
D25	99.1	101.1	97.04	49
D50	99.15	100.75	97.55	95.8
D100	97.6	92.3	102.9	197.33
D200	102.5	103.8	101.14	513.7
Average	99.6	99.5	99.65	214

50, 100, and 200, respectively. For lab samples, the mean average run time was 49, 95.8, 197.33, and 513.7 seconds per image for densities 25, 50, 100, and 200, respectively. The overall average processing time was 88.95 and 214 seconds per image for digital images and lab samples, respectively (Tables 2 and 3).

Discussion

Using AI for counting and identifying *Drosophila melanogaster* individuals

FlydAI is an AI that automates the counting and identification of large populations of wild and white morphs of *D. melanogaster*, minimising the labour of the manual procedure. Following the approach presented here, we counted and identified wild and white morph flies in samples up to 200 individuals: 99.89% of individuals in the samples were counted, and all of them were assigned the correct phenotype in 78.81 seconds of average run time. Based on these results, the use of FlydAI potentially could help to reduce labour time for these routine tasks in a *D. melanogaster* lab.

When input images are larger than 1094 × 768 pixels, FlydAI divides them in smaller fragments, and these are processed separately by the AI, processing a few individuals at a time. We tested different combinations of fragments as inputs to reach the different densities. We found the accuracy and the processing time were not significantly affected by sample size (see Table 1), indicating that the number of flies to be counted and identified can be increased without hindering these parameters. As mentioned in the section, Development of AI for image recognition, in this paper, a fly that overlaps (is partially present) in two images as a result of the AI cropping process can be misclassified or classified twice. The grid card provided (Supplementary material 1) allows the user to place the anaesthetised flies within the squares to help make sure all of them are classified correctly. The print-ready sample card can be easily transformed onto a sticky card to facilitate the process of image capturing.

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Efficiency of human workers *versus* FlydAI

For experienced human workers, the rate of accurate estimation of number and phenotype of individuals was almost 100% in all cases, although a slight tendency was observed when viewing digital images to overestimate the number of individuals in samples of up to 100 individuals and to underestimate this number in samples at a density of 200 individuals. When observing samples under a microscope, workers showed an opposite tendency, undercounting flies at lower density and overcounting flies at the highest density (200 flies). However, all counting errors by workers were minor.

The preparation of the samples to be processed by both FlydAI and human operators does not vary significantly: in both cases, it is necessary to anaesthetise the flies, place them on a solid surface (cardboard grid or watch glass, depending on the case), and position them for observation (with a DLSR camera or dissecting microscope, respectively). Apart from that, human processing time at low or moderate densities was similar to FlydAI for digital images (Table 2) or even lower for lab samples (Table 3). As the number of individuals per sample increased, the human processing time dramatically increased, whereas the AI run time remained constant. On average, a human worker was 14.75% slower than FlydAI when processing digital images and 64.6% slower when processing lab samples. For the samples with higher densities used in this study (200 flies), processing time was 513.7, 153.09, and 76 seconds per image for lab samples, digital images, and AI, respectively. This suggests that a human worker would take twice as long as FlydAI would to process a digital image. The time spent by a human operator to process a lab sample would be almost seven times greater than the time spent by the AI (see Tables 1–3). It is reasonable to think that, as we increase the number of samples processed throughout a workday, the efficiency of a human worker would decline due to fatigue, whereas artificial intelligence will maintain steady performance. This makes FlydAI an effective alternative to processing by bench workers.

Automated recognition methods

Most traditional manual methods to estimate size population of insects are trap-based (pitfall traps, emergence traps, malaise traps, pan traps, light traps, *etc.*). In some other cases, insects become fixed to a physical surface (*i.e.,* on spot cards, sticky traps, and fly ribbons; Gerry 2020) and then are counted by a human worker. In automated approaches, the surface bearing the insects or insect marks is photographed, and an AI tool processes the data (Gerry *et al.* 2011; Ding and Taylor 2016; Zhong *et al.* 2018; Zhu *et al.* 2018). These image-recognition methods are used commonly to count flies (Gerry 2020), which may be difficult to identify and count when observed alive due to their reduced size and their unpredictable, erratic motility. Those characteristics complicate their identification, increase the likelihood that an individual fly is counted multiple times, and make obtaining clear pictures of them in flight difficult. Even though video-monitoring devices are normally intended to count bigger-sized animals (Pérez-Escudero *et al.* 2014; Bentley *et al.* 2023), some early efforts have been used to detect insects in motion (Bjerge *et al.* 2021, 2023; Kirkeby *et al.* 2021; Tannous *et al.* 2023; Roy *et al.* 2024).

For pest control, the introduction of electronic and automatic traps that combine hardware to collect insects and software to send and process data offsite is increasingly common (Potamitis *et al.* 2018; Preti *et al.* 2020; Plá *et al.* 2021; Diller *et al.* 2023). Some such tools have already been commercialized: for example, Trapview (https://trapview.com/) developed a device that automatically collects and sends data to be analysed by AI, and RapidFLY (https://rapidaim.io/) provides a trap equipped with a sensor that is able to detect the presence of fruit flies.

In recent years, various AI have been developed and emerged. Some, such as Google's Vertex AI, have powerful image-recognition capabilities. However, they still all have drawbacks: they operate under pay-as-you-go models with costs quickly escalating, they have limited customisability, and they depend on computational resources or Internet availability.

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Ferreira Lima *et al.* (2020), Høye *et al.* (2021), and Schneider *et al.* (2023) show that there are many existing applications to automate the estimation of size population and the type of insects. However, most methods cited by those authors discriminate among individuals belonging only to different orders, genera, or, at best, species, and none of the tools has been trained to differentiate *Drosophila* morphs. Although this limitation might be useful for pest control, it lacks sufficient accuracy for laboratory work. The FlydAI, on the other hand, accurately recognises and counts *D. melanogaster* mutants using a digital image as source. Once the individuals are fixed onto a surface, only a device able to capture macro images of a minimum of 1094 × 768 pixels is needed.

Criticisms

The high percentage of success of FlydAI determined in the present study may be argued as due to the high-quality images used for the experiments. The images were taken under controlled light and focus conditions, simulating a feasible lab situation. To test the AI usability and replicability under different conditions, we altered a set of images. Adding noise to the images increases the AI's resilience to artefacts similar to those produced in poor-light conditions. Our aim was to train a tool that can be used even when image-acquisition conditions are suboptimal – for example, during field trips or in facilities where specialised equipment is unavailable. In our tests, FlydAI success rate in assessing the correct number of flies was near 100% for all densities tested when images had up to 15% artificial noise added. The success rate dropped to 75% when images with 30% noise were used. No significant differences in success rate were observed as the density of individuals increased. Almost all of the flies detected in images with up to 5% noise were phenotyped correctly. As the level of noise increased in the images, the AI tended to misidentify white individuals, categorising them as wild-type flies: FlydAI overestimated wild-type flies by 15.2% and 47.74% for images with 15% and 30% noise, respectively; and it underestimated white flies by 17.77% and 96.33% for images with 15% and 30% noise, respectively).

To prevent the model classifying only flies presented at the same scale and to increase the efficiency of the AI, different image sources (from a DLSR camera and a digital microscope, with different levels of magnification and detail) were used for training. However, regardless of the technical and environmental conditions in which our tests were conducted, it is highly unlikely that in real *Drosophila* experiments, users will feed the AI model with images with the level of artificial noise we added for the present study. Most commonly used devices that take photographs are capable of producing high-quality images. In fact, more and more research demonstrates the application of images taken with mobile phone cameras in diverse experimental contexts (Ozcan 2014; Switz *et al.* 2014).

To further prove the efficiency of the FlydAI, we applied a test using a set of images downloaded from the Internet. The lack of a sufficient number of images exclusively containing wild and white *D. melanogaster* flies did not permit robust conclusions, but FlydAI detected both phenotypes with the expected accuracy even when *D. melanogaster* drawings were used (see Data availability).

Final remarks

The data presented here indicate that FlydAI is an accurate, fast, and reliable tool for automated phenotyping of two different morphs of *D. melanogaster* (wild and white) and for counting large number of individuals and that using FlydAI significantly reduces processing time for counting and identification. In terms of efficiency and time employed, the tool surpassed the performance of both other methods and experienced human operators. Thus, FlydAI is a viable alternative to human workers in routine lab tasks, especially when the quality of the images is high or moderate (up to 15% noise) and the number of individuals is high (200 or higher). The protocol we present in this study could be used to benefit researchers in fields such as population biology, ecology, and

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evolution, where they must deal with a large number of individuals. The FlydAI is also customisable for different phenotypes and species and requires a minimal supervision by specialised operators. Programs, scripts, sample images, and the protocol have been made available in a GitHub repository, and users can easily customise the methodology by training the AI with their own images. A step-by-step protocol and the AI can be accessed *via* a Google Colab virtual machine. Furthermore, FlydAI is affordable; TensorFlow Lite is specifically designed for developing models on microcontrollers and other mobile and edge devices. This allows FlydAI to be run using a 2.2-GHz processor, making possible the use of a low-cost Raspberry Pi (model 4b 8-Gb RAM) compact computer (Raspberry pi OS 11 64-bit; Raspberry Pi Foundation, Cambridge, United Kingdom) to process the AI-generated files. This means that the methodology presented here is portable and can be used outdoors or in facilities where no specialised instruments are available.

Prospective work

The protocol provided in the present study (see Data availability) easily allows *ad hoc* training of the AI for the recognition of not only other *D. melanogaster* mutants but can also be customised to recognise a wide range of other animal species. Although FlydAI was designed exclusively to facilitate routine lab tasks dealing with estimating the number of white and wild *D. melanogaster* individuals, with the proper adjustments, it could be used in the field or in different types of facilities to routinely record fly activity. The second section of the online protocol (see Data availability) outlines how to train and use the model using other images of other materials or insects.

The success of the proposed methodology relies on the availability of images containing a representation of individuals in a population. The workflow suggests that multiple operators can capture the images onsite, with subsequent data processing centralised offsite to reduce travel time and expenses of specialised workers. One possible scenario would be for the digital images to be processed by human workers. However, as shown in the present paper, FlydAI still is a more efficient solution.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.4039/tce.2024.36.

Acknowledgements. The authors thank the Fundación Española para la Ciencia y la Tecnología, FECYT [FCT-21-17334] for funding this work. Funding for open access for this paper was provided by the Universidad de Granada.

Competing interests. The authors declare that they have no competing interests.

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Cite this article: Gálvez Salido, A., de la Herrán, R., Robles, F., Ruiz Rejón, C., and Navajas-Pérez, R. 2024. Automatic counting and identification of two *Drosophila melanogaster* (Diptera: Drosophilidae) morphs with image-recognition artificial intelligence. The Canadian Entomologist. https://doi.org/10.4039/tce.2024.36.

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