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Author(s): Jeffrey D. Wells, Ph.D., Matthew DeGennaro, Ph.D.

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FINAL REPORT

2019-DU-BX-0013. Germ-Line Transformation of Forensically Important Flies

Principal Investigator: Jeffrey D. Wells, Ph.D.

Co-Principal Investigator: Matthew DeGennaro, Ph.D.

PI Email Address/Phone/Affiliation: jedwell@fiu.edu / 305-348-1320/
Department of Biological Sciences, Global Forensic and Justice Center

Name of Submitting Official: Roberto M. Gutierrez
Submitting Official Email Address/Phone: gutierrr@fiu.edu / 305-348-2494
Recipient Organization: Florida International University
11200 SW 8th Street, Miami, Florida 33199

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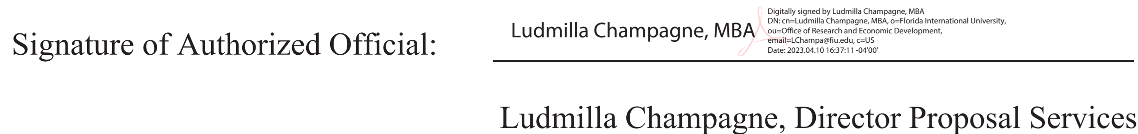

Ludmilla Champagne, Director Proposal Services

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Summary of the Project

Major Goals and Objectives

The goal of this project was to develop a method for genetically engineering carrion flies to produce a fluorescent protein (in all stages and tissues) and without altering development rate. The potential, future, practical application is that this will make it much easier to assess the accuracy of insect-based estimates of time since death.

The most common forensic entomological analysis is estimating the age of a fly larva, i.e., a maggot, associated with a corpse, because that age can provide a minimum postmortem interval (PMI_{min}), or "Dead for not less that X amount of time." The process involves a comparison, using one of several mathematical techniques, of a corpse maggot (or other life stage) to maggots of known age that were reared in a laboratory (e.g., Tarone and Foran 2008, Ieno et al. 2010, LaMotte and Wells 2015). Forensic insect age prediction is highly accurate under carefully controlled laboratory conditions (e.g., Wells and LaMotte 1995, LaMotte et al., 2017), or in artificial containers held outdoors (Faris et al. 2020) but there has been little empirical validation of maggot age estimation performance under death scene conditions. Until now, a practical barrier to such a validation experiment was that it is extremely difficult to know the true age of a maggot on an experimental (i.e., "body farm") corpse or large animal carcass. If the true age is not known, then the accuracy of the age-estimation method cannot be assessed.

If a carrion insect placed on the experimental corpse were labeled, e.g., dyed a different color, so that it could be identified from among the hundreds of thousands of similar larvae, the validation experiment problem would be overcome. Eggs laid within a brief time window from a laboratory colony could be added to the corpse population and would be of known age when later recovered. However, the label cannot be a surface dye because insects periodically shed

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their exoskeleton as they grow, and it could not be an ordinary dye (or analogous substance such as rubidium) injected into the egg because this would be too metabolized and/or diluted to detect after the growing insect became thousands of times larger. We therefore endeavored to label carrion insects by genetically engineering them to produce a marker, in the form of a fluorescent protein, in all tissues throughout life. We proposed two major research objectives.

1) The first objective was to use germ-line transformation techniques to produce laboratory strains of *Phormia regina* and *Chrysomya megacephala* that express a fluorescent protein. "Germ-line" refers to the cells that produce egg or sperm, thereby ensuring that all descendants of the transformed insect will fluoresce when illuminated by the correct wavelength of light.

2) The second, was to determine if the genetic transformation, which potentially can damage the organism, had altered the growth rate of the insect. In other words, does the transgenic strain develop at a rate different from the original untransformed strain? If not, the transgenic insects should be suitable for field validation of insect age estimation methods.

Changes in the Original Proposed Experimental Design

We modified the proposed plan in response to two unanticipated sources of difficulty. The first difficulty was the closing followed by partial reopening of our research laboratory during the first six months of the pandemic. The second difficulty was the much slower than expected progress in our attempts to transform the insects once normal laboratory access resumed. To deal with these setbacks we: 1) concentrated our efforts on just one of the study species, *P. regina*, to increase the probability of transformation success, and 2) developed a *P.*

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regina genome and transcriptome to support our efforts to design a successful transformation protocol for that species.

Laboratory and Statistical Analysis Methods and Results

OBJECTIVE 1

The study animal is the forensically important blow fly *Phormia regina*, potentially the species most commonly used as evidence in a North American death investigation (Andere et al. 2016). Our laboratory colony, conventionally referred to as the wild-type indicating no artificial change to the genome, originated from West Virginia (Roe 2014).

All genome modification procedures involved microinjection of embryos (Figure 1) targeting the pre-blastoderm stage, the initial stage of insect development when cell membranes have not yet formed and the nuclei are more exposed to manipulation. Freshly laid eggs obtained from flies in a laboratory cage were injected with synthetic or recombinant plasmid DNA that varied according to the transformation approach being tried (see below). **A full description of the technical details of all transformation experiments is found in MacInnis (2023).**

Injected embryos were held until they became larvae, screened for fluorescence on a Leica fluo III microscope with X-cite 120LED illumination system using a DsRed filter (Leica ET DSR for MZ FL III) then reared to adults. All resulting adults were retained for the crossing procedure (breeding experiments), although any fluorescence was noted. Up to two generations of outcrossing with the wild-type (if there was any sign of fluorescence) was followed by incrossing (breeding fluorescent with fluorescent) the G3 generation to produce a homozygous line (Figure 2).

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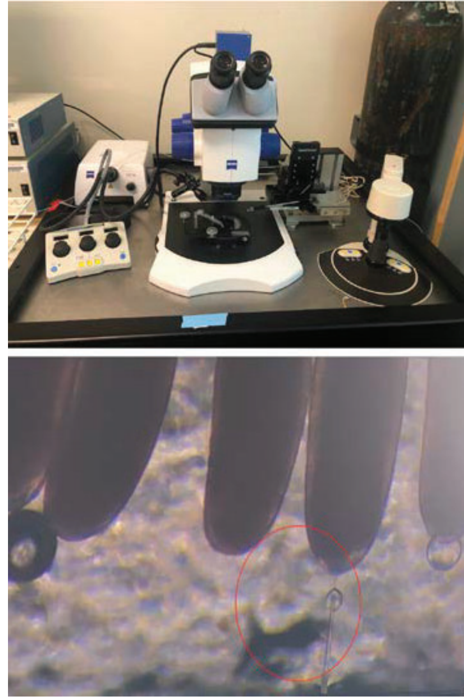

Figure 1. *P. regina* embryos were injected at the posterior end (see second egg from right) with a Zeiss SteREO Discovery.V8 (Zeiss, Jena, Germany) paired with a Sutter Instrument® Xenoworks micromanipulator and digital microinjector model (Sutter, Novato, CA) and using a borosilicate needle.

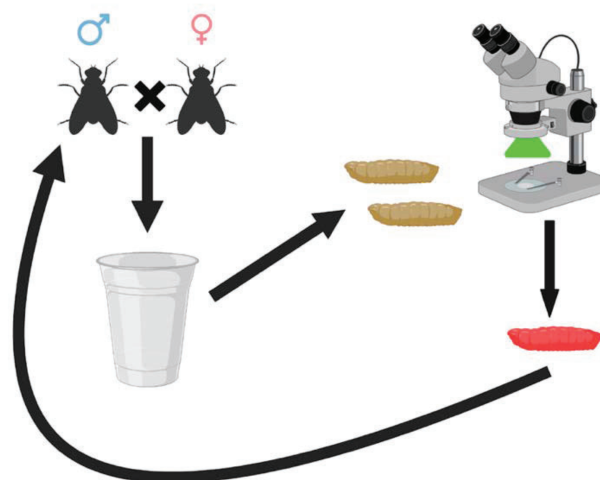

Figure 2. Diagram of the crossing and screening procedure. Flies were crossed at a 1:3 ratio of injected to wild-type, except in the final incross which was a 1:1 ratio. The larvae were removed as L3 and screened using a fluorescent microscope, removing any fluorescent individuals for the next outcross. The third generation of larvae were incrossed to produce homozygous individuals.

Four laboratory transformation protocols were attempted, one of which was successful.

The unsuccessful attempts will be very briefly described.

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

CRISPR can be used to insert a transgene at a known location in the genome (Wright et al. 2016). Using our *P. regina* genome (see below), we designed a CRISPR/Cas9 system paired with T2A in the target Actin5C gene and microinjected 2,095 embryos. The result was high embryo mortality and no evidence of transformation.

PiggyBac Transformation Using a Donor Plasmid with an Artificial Eye Promotor 3xP3

The *piggyBac* approach involves the microinjection of two DNA plasmids. The donor plasmid contains the transgene and the helper plasmid contains the gene for the transposase that will insert the transgene at one or more, uncontrolled, locations in the genome (Figure 3).

We injected 1092 embryos with a helper plasmid driven by a promotor of the constitutively expressed heat shock protein hsp83 derived from the blow fly *Lucilia cuprina* and a donor plasmid with the fluorescent protein gene DsRed Express driven by an artificial eye promotor 3xP3, known to have very high levels of gene expression activity. Although a heat shock protein serves to protect against high temperature and its activity is enhanced by heat in the original organism, published *piggyBac* protocols with the hsp83 promotor do not include an elevated temperature step (a heat shock) so we did not employ one. Post-injection survival to adult stage was high (14%) but we observed no evidence of germ-line transformation.

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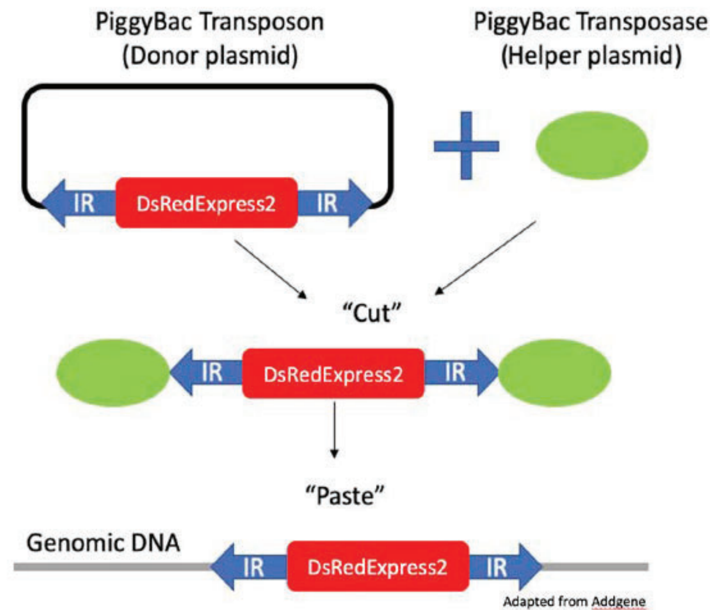

Figure 3. A schematic representation of the *piggyBac* approach to inserting a foreign gene in the genome. In this representation the transgene DsRedExpress2 is that used in our successful generation of a transgenic line of *P. regina*.

PiggyBac Using the hsp83 Promotor on Both Plasmids and No Heat Shock

This experiment used the same donor and helper plasmid design that successfully transformed two *Lucilia* spp. blow flies (Concha et al. 2011). As mentioned above we did not include a heat shock step because none was mentioned by Concha et al. (2011). We injected 977 embryos with 6% survival to the adult stage but we observed no evidence of germ-line transformation.

Successful *piggyBac* Germ-Line Transformation of *P. regina* Using the hsp83 Promoter on with a Heat Shock Step

1391 embryos were injected as in the previous attempt, but the injected embryos were heated to 37°C for 30 minutes and larvae were similarly heat shocked 16 hours before screening to promote hsp83 activity and expression of DsRed. 9% of injected individuals survived to the

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adult stage. **An outcross between an injected male and wild-type females produced eight transgenic offspring (i.e., showing fluorescence throughout the individual), six of which survived to the adult stage (5 males and 1 female).**

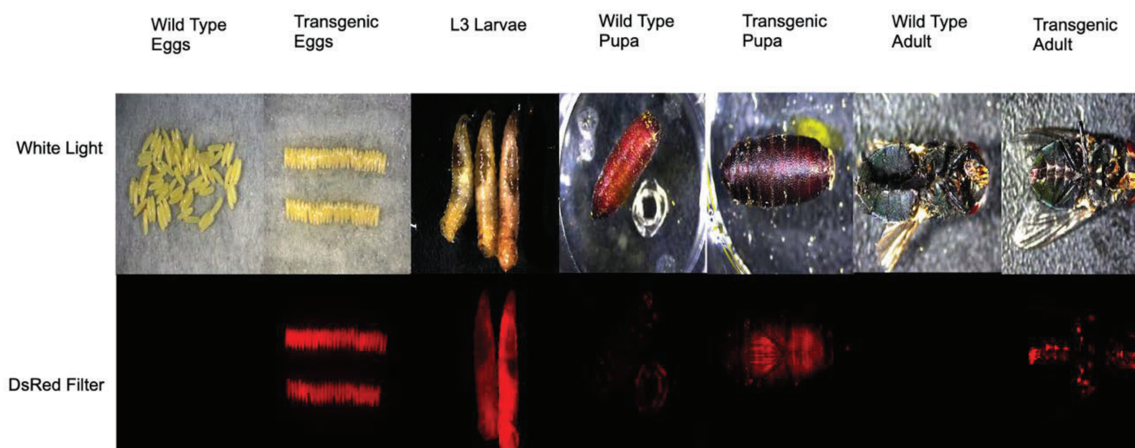

Figure 4. A side-by-side comparison of transgenic and wild-type eggs, larvae, pupae, and adults. Top row specimens were illuminated with white light and photographed without an optical filter. Bottom row specimens were illuminated by laser green light and photographed with a filter for DsRed maximum emission. The L3 larvae show that the heterozygote (middle) is both less pink in white light and less red under green excitation compared to the homozygote (right).

Because only one injection including the heat shock succeeded, this by itself is weak evidence that the heat shock made the germ-line transformation more efficient. However, compared to the *piggyBac* without a heat shock, we also observed a considerable increase in the number of larvae with mosaic fluorescence, i.e., in isolated parts of the body therefore not in the germ line. This suggests that the activity of hsp83 was increased by heat shocking the injected embryos.

The resulting fluorescent G1 progeny and subsequent generations displayed constitutive and uniform fluorescence in the body and throughout the life stages (Figure 4). The homozygous individuals from the G3 incross resulted in larvae that showed brighter fluorescence to the naked eye than the heterozygous individuals. Additionally, the larvae of both genotypes showed a pink

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color even under white light, with the homozygous individuals showing a darker shade of pink. The fluorescence is seen across all life stages from egg to adult. Fluorescence in eggs of a transformed female was observed even when the hatched larvae did not show fluorescence, e.g., when crossed with a wild-type male, suggesting that either the DsRed protein or mRNA is provisioned in the egg by the mother.

Characterization of the DsRed Transgene Insertion Site within the *P. regina* Genome

The transgene occurs at a single genome location

Genomic DNA was extracted from each of the six fluorescent G1 adults and sent for Illumina technology sequencing (35X coverage) by the Oklahoma Medical Research Foundation NGS Core (Oklahoma, USA). The resulting data were assembled to cover major continuous regions of the genome of each specimen. A single DsRed gene sequence was then found within each genome, and the sequences on each side of the insertion were the same for each fly. Therefore, the transgene was inserted at only one genomic location in all transformed flies.

The transgene is located in a intergenic region far from any expressed gene, and therefore unlikely to disrupt gene function in the fly

Using our *P. regina* transcriptome data (see below) we mapped messenger ribonucleic acid (mRNA) sequences to the combined genome of all six transgenic flies. mRNA is the product of a gene, so any match with the genome reveals the presence of a gene. The gene closest to the insertion site was >6000 DNA bases away. Therefore, no original gene was disrupted by the insertion of DsRed.

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Sequencing, Assembly, and Annotation of a High-Quality *P. regina* Genome

To support the gene insertion efforts just described, we extracted DNA from a single *P. regina* adult male. This was sent to a commercial DNA sequencing service. The resulting files representing short pieces of DNA were assembled into a more complete genome, after which the individual genes were identified or "annotated," using standard software based on genes known from closely related organisms and based on the *P. regina* gene transcripts described in the next section. (Figure 5).

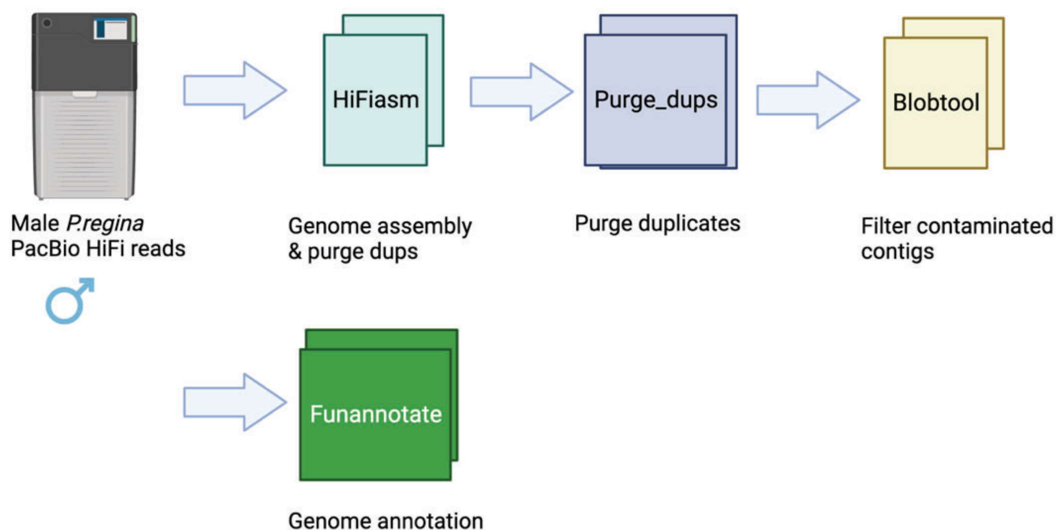

Figure 5. Schematic workflow of the *P. regina* genome analysis pipeline. The sequence data files obtained from PacBio circular consensus sequencing were first assembled into a genome, followed by Blobtool analysis to identify erroneous data (e.g., duplicates and non-*P. regina* contamination). The filtered contigs were then annotated through a Funannotate pipeline.

This genome is now about 95% complete (Figure 6), and much higher quality compared to what was previously available for *P. regina* (Andere et al. 2016).

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	Genome assembly
CDS transcripts	18284
CDS 5' to 3' UTR	6257
Total exons	83258
Average exons length	405.61
Secretion	2133

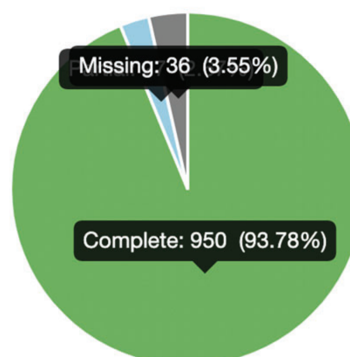

Fig 6. Summary of *P. regina* genome annotation showing degree of completeness compared to other publicly available arthropod genomes. (https://gvolante.riken.jp/)

Transcriptome Analysis to Identify *P. regina* Genes

RNA was extracted from one virgin adult male and one virgin adult female *P. regina* using an QIAGEN RNeasy Plus extraction kit (MA, USA). The concentration of RNA samples was assessed with an Invitrogen Qubit Fluorometer using a Qubit RNA HS Assay kit (MA, USA) and the integrity of extracted RNA was assessed with a Bioanalyzer with Agilent RNA 6000 Pico chip. mRNA molecules were "reverse transcribed" to make the matching DNA, which was then sequenced and interpreted in a manner similar the previous section.

OBJECTIVE 2

The purpose of this project was to assess the effect of the genomic transformation on the growth rate of *P. regina*. Moreover, we wanted to know if development rate was altered enough to reduce the accuracy of age prediction performance rather than just a statistically significant effect that might not have any practical implication (Wells and LaMotte, 2017).

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In a future field validation experiment, age-prediction performance will apply to using wild-type growth-rate data from the lab to estimate the age of transgenic individuals in a natural setting (Because this mimics current forensic entomology practice.). If such age predictions are sufficiently accurate when both genotypes developed under the same environmental conditions, then any greater error in the analysis when the transgenic larvae are in a corpse can be attributed to corpse conditions and not the genomic transformation.

The following is an abbreviated description of the experiment. Complete details are in MacInnis (2023).

Eggs were obtained over a 2-hour interval from wild-type and homozygous laboratory colonies, and from lab colonies of homozygous females mated with wild-type males, i.e., the eggs were heterozygous for the transgene. Newly hatched larvae were place in cups, about 30 per cup, with chicken liver for food. Each cup was then placed within a plastic box with a screened air hole and sawdust for a pupation medium (Figure 7). Boxes with larvae were then held in an incubator at 27.5 ± 0.1°C with a 16:8 light:dark cycle.

For each of three replicated growth experiments, containers of all three genotypes were set up for sampling at ages 18, 24, 30, 36 ,42, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180, and 192 hours. Sampling involved removing the entire cohort of insects in a container. Within one experiment there were two containers for each age. Therefore, the total number of rearing cups was 3 genotypes X 3 separate experiments X 2 replicates per experiment for each of the 18 sampling times giving a total of 324 rearing containers.

Sampled larvae were immersed in 85-90 °C water for 30 seconds or until elongated then preserved in 70% ethanol for storage and larval instar determination based on posterior spiracle morphology. Third larval instars were classified as feeding if still on the food and postfeeding

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Figure 7. Rearing containers consisting of plastic boxes with paper cups containing liver and blow fly larvae over wood shavings. The paper towels on top were removed after 60 hours to facilitate larval wandering and pupation.

if in the sawdust. Once pupae were noticed in the container, they were placed in plastic 24 well plastic plates with a single individual per well. The pupae were observed every 12 hours and were classified as either pupa without respiratory horns present, pupa with respiratory horns present, or adult. The date of transition to each phase was recorded for each individual and the numbers at each stage were recorded for each time point on a table format. In summary, each sampled insect was classified as one of seven developmental stages: first larval instar (L1), L2, L3 feeding, L3 wandering, pupa no visible respiratory horn, pupa visible respiratory horn, adult.

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A table of the pooled wild-type stage data with the rows corresponding to age and the columns corresponding to stage was used to make 95% predictions of age for a mystery specimen of a given life stage following the method of LaMotte et al. (2017) (Table 1).

Table 1. Counts of wild-type individuals by stage and age. The shaded cells were rejected at the 5% statistical level for a hypothesis of age for a given stage. Therefore, the unshaded cells in a column are the 95% prediction interval for that life stage. For example, an L2 individual, age unknown, is predicted with 95% confidence to be between 36 and 72 hours old, because L2s were too rarely observed (in this example, never) at other ages.

Hours	L1	L2	L3F	L3W	PNH	PH	A
18	167	0	0	0	0	0	0
24	167	0	0	0	0	0	0
30	176	0	0	0	0	0	0
36	139	23	0	0	0	0	0
42	8	140	0	0	0	0	0
48	5	164	0	0	0	0	0
60	1	164	6	0	0	0	0
72	0	21	140	0	0	0	0
84	0	0	151	8	0	0	0
96	0	0	107	52	0	0	0
108	0	0	99	58	0	0	0
120	0	0	31	132	0	0	0
132	0	0	11	155	1	0	0
144	0	0	0	100	33	21	0
156	0	0	0	59	42	111	0
168	0	0	0	1	71	216	0
180	0	0	0	4	26	394	0
192	0	0	0	1	19	549	0
204	0	0	0	0	18	549	0
216	0	0	0	0	18	549	0
228	0	0	0	0	18	549	0
240	0	0	0	0	18	549	0
252	0	0	0	0	17	457	93
264	0	0	0	0	17	357	193
276	0	0	0	0	11	77	479
288	0	0	0	0	11	30	526
300	0	0	0	0	10	28	529
312	0	0	0	0	10	27	530

When the wild-type age rejections are superimposed on similar tables for the two transgenic genotypes Tables 2 and 3), any insect in a shaded cell is an individual whose true age does not fall within the age prediction, i.e., it is an age prediction error. Because the age

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Table 2. The heterozygous genotype development data with the wild-type statistical age rejections from Table 1 superimposed as either a gray or blue cell. This shows the performance of using wild-type *P. regina* data to predict the age of a transformed *P. regina*. Counts in blue cells are individuals whose true age was not captured by the prediction intervals, i.e., it represents an age-prediction error. 99.7% of age predictions were correct in that they fell within the confidence interval.

	Heterozygotes						
Hours	L1	L2	L3F	L3W	PNH	PH	A
18	182	0	0	0	0	0	0
24	183	0	0	0	0	0	0
30	167	0	0	0	0	0	0
36	183	2	0	0	0	0	0
42	11	161	0	0	0	0	0
48	3	231	0	0	0	0	0
60	0	191	4	0	0	0	0
72	0	4	222	0	0	0	0
84	0	0	160	12	0	0	0
96	0	0	128	55	0	0	0
108	0	0	106	61	0	0	0
120	0	0	48	140	**2**	0	0
132	0	0	1	152	**13**	**4**	0
144	0	0	0	142	37	17	0
156	0	0	0	21	104	99	0
168	0	0	0	**4**	88	237	0
180	0	0	0	0	23	509	0
192	0	0	0	**2**	20	699	0
204	0	0	0	0	18	701	0
216	0	0	0	0	18	701	0
228	0	0	0	0	18	701	0
240	0	0	0	0	18	701	0
252	0	0	0	0	18	535	166
264	0	0	0	0	18	367	334
276	0	0	0	0	13	78	629
288	0	0	0	0	8	29	682
300	0	0	0	0	7	27	685
312	0	0	0	0	7	25	687

Table 3. The homozygous genotype development data with the wild-type rejection ages superimposed as in Table 2. 99.8% of age predictions were correct.

	Homozygotes						
Hours	L1	L2	L3F	L3W	PNH	PH	A
18	141	0	0	0	0	0	0
24	161	0	0	0	0	0	0
30	190	0	0	0	0	0	0
36	148	28	0	0	0	0	0
42	3	145	0	0	0	0	0
48	0	152	0	0	0	0	0
60	0	123	9	0	0	0	0
72	0	6	146	0	0	0	0
84	0	0	160	10	0	0	0
96	0	0	190	17	0	0	0
108	0	0	87	53	0	0	0
120	0	0	60	99	0	0	0
132	0	0	3	191	**6**	0	0
144	0	0	**1**	73	59	24	0
156	0	0	0	22	95	105	0
168	0	0	0	**4**	83	285	0
180	0	0	0	**5**	32	501	0
192	0	0	0	**1**	28	651	0
204	0	0	0	0	24	655	0
216	0	0	0	0	24	655	0
228	0	0	0	0	24	655	0
240	0	0	0	0	24	655	0
252	0	0	0	0	22	485	172
264	0	0	0	0	13	225	441
276	0	0	0	0	7	87	583
288	0	0	0	0	7	26	643
300	0	0	0	0	7	22	650
312	0	0	0	0	7	21	651

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prediction is in the form of a 95% confidence interval, the standard for acceptable prediction performance is that 95% or more of predictions be correct. This was the case when predicting the age of both heterozygous and homozygous strains (Tables 2 and 3). **Our research showed that the transgenic strain development rate did not appear to have been altered to a practical degree. Therefore, these insects are suitable for validation studies of forensic entomology age estimation methods.**

Expected Applicability of this Research

Most importantly, we or any other research group can now use these transgenic *P. regina* to assess the real-world accuracy of the most common forensic entomological analyses. Furthermore, any scientist interested in the biology (and not necessarily the forensic uses) of blow fly larvae now can track an individual larva on a corpse, because it can be seen with a blue flashlight while wearing orange- or red-lens glasses. This makes it possible for the first time to observe what an individual maggot does on a corpse and thereby to discover if these seemingly simple creatures have more complex behavior than is assumed. Does a larva thermoregulate by moving in and out of a maggot mass? Do maggots aggregate in sibling groups as is apparently done by adults (Picard and Wells 2009)?

Limitations of This Research

Future field validation experiments will be constrained by the fact that the transgenic insects must not be allowed to escape. Of course, for a university scientist any research on

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transgenic organisms is supervised by an Institutional Biosafety Committee that must approve the experimental design and be confident that the transgenic organisms will be safely contained.

There is a trade-off between the degree to which validation experiments can approximate a real death scene and the need to contain the transgenic insects. Wild flies must have access to the corpse to establish a dense mixed-age and mixed-species larval population. At the same time there must be an impenetrable barrier to prevent larval escape and, if there is any chance the transgenics will reach the adult stage, newly emerged transgenic adults.

We believe that maximizing naturalness for the final validation experimental design, involving human donor corpses or domestic pigs, environmental conditions plausibly matching a real death scene, and the least amount of barrier to wild insect access, while preventing transgenic insect escape, can only be understood by performing preliminary experiments. In other words, we intend to start with smaller carrion and relatively strict containment, then increase wild insect access while monitoring containment. A likely approach, mimicking a corpse in a garbage bin (Reibe et al., 2008), is shown in Figure 8. We have found that a sleave fashioned from pantyhose prevents the escape of larvae that crawl up the side of the bin. The sleave can be initially open to allow fly access. After transgenic eggs are added to the carcass the sleave will remain open to allow the return of wild flies, likely to be scared away by the activity of adding the transgenic eggs. Once the visiting fly population is restored, we will quickly close the sleave, trapping flies that should continue to deposit eggs. Therefore, the transgenic maggots will be in the presence of both older and younger wild larvae, a more natural condition than if they are always the youngest on the carcass.

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Figure 8. A plastic garbage bin sealed with a sleave made from pantyhose. We found that reared in an animal carcass placed on a layer of wood shavings in the bin could not penetrate the sleave after crawling up the side of the bin.

At the appropriate time for recapturing the transgenic larvae, the entire bin could be placed in a freezer to stop development and kill all the insects. Killed transgenic larvae still fluoresce.

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Participants and Other Collaborators

Florida International University

Jeffrey D. Wells, Ph.D., Principal Investigator; Matthew DeGennaro, Ph.D., Co-Principal Investigator, Andre Costa da Silva, Ph.D., Postdoctoral Research Scientist, Anthony Bellantuono, Ph.D., Research Assistant Professor; Amber MacInnis, M.S., Ph.D. Student; Sheng-Hao Lin, M.S., Ph.D. Student; Maria Mena, Undergraduate Student; Matthew Cueto, Undergraduate Student.

LSU Health New Orleans

Lynn R. LaMotte, Ph.D., Statistical Consultant.

Artifacts

Publication

MacInnis AE. 2023. *A Genetically Marked Maggot to Better Understand Corpse Ecology*. Ph.D. Dissertation. Florida International University.

Scientific presentations that included this project

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Lin S-H, Bellantuono A, Costa de Silva A, Lopez K, Wells J, DeGennaro M. Producing an improved *Phormia regina* genome: A resource for the development of new molecular genetic tools for forensic entomology. Florida International University 10th Annual Forensic Science Symposium, held virtually June 7-11, 2021.

MacInnis A, Wells J. Genetic engineering of forensically important insects. Florida International University 10th Annual Forensic Science Symposium, held virtually June 7-11, 2021.

MacInnis A, Costa-da-Silva A, Ballantuono A, DeGennaro M, and Wells J. “Generating fluorescent blow fly strains to aid in validation of postmortem interval estimates.” Poster, FIU Annual Biomolecular Sciences Institute Research Symposium, June 22, 2022. [Awarded second place in the graduate student competition.]

Wells JD. Molecular biology applications for forensic entomology. Academic department seminar, Institute of Medical Molecular Biology, Universiti Teknologi MARA, Malaysia.

MacInnis A. Generating fluorescent blow fly strains to aid in validation of postmortem interval estimates. Joint meeting of the Entomological Societies of America and Canada, Vancouver, BC, November 14, 2022 [First place student award, Medical, Veterinary, and Urban Entomology category]

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Wells JD. Molecular biology applications for forensic entomology. Academic department seminar, Department of Biology, Indiana University Purdue University Indianapolis, January 20, 2023.

MacInnis A. Germ-line transformation of forensically important flies. NIJ Forensic Science R&D Symposium, Orlando, FL, February 14, 2023.

MacInnis A, da Costa Silva AL, Bellantuano A, DeGennaro M, Wells J. Generating fluorescent blow fly strains to aid in validation of postmortem interval estimates. American Academy of Forensic Sciences annual conference, Orlando, FL, February 17, 2023.

Our transgenic insects will be made available to the scientific community

We will maintain a colony of the transgenic *P. regina* and will provide live specimens, provided that a Material Transfer Agreement is in place, in response to a reasonable request from a research scientist.

Development rate data for the wild-type, heterozygous transgenic, and homozygous transgenic *P. regina* will be publicly available

We will include our raw data as a supplementary file with the scientific journal manuscript we anticipate submitting before June, 2023.

Graduate education

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This grant supported two Ph.D. students. Amber MacInnis successfully defended her dissertation on April 3, 2023. On the strength of this work Dr. MacInnis was offered and accepted a postdoctoral research position at Texas A&M University, College Station. Sheng-Hao Lin advanced to candidacy and successfully defended his research proposal.

Dissemination activities

We presented our results to eight scientific audiences (see above). The germ-line transformation and transgenic strain evaluation portion of the research is included in a Ph.D. dissertation (see above) that soon will be visible through a standard scientific literature search. We will submit a scientific manuscript on the creation and evaluation of the transgenic strain before June 2023. We will submit a second manuscript on the genomic and transcriptomic work before September 2023.

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