Morphometrics in Third Instar Larvae of Blow Fly Species (Diptera: Calliphoridae)

Lauren Quatroche
Mentor: Dr. Trevor Stamper

Introduction

Blow flies (Diptera: Calliphoridae) are a group of insects of great forensic importance, due to Larvae development and species succession being an indicator of postmortem interval (Nuñez & Liria, 2016). However, larval identification of species can be difficult, and while the easiest method is to rear the larvae and wait for them to emerge as adults, it is not always possible. Larvae may fail to develop and die, or are simply already dead and preserved when presented to the entomologist (Erzinçlioğlu, 1985). Taxonomic keys for larvae are available, but considering the level of accuracy ideal for testimony, it may be useful to know the typical variation in size of certain morphological structures for a species, making it possible to ascertain species identification via measurement and statistics.

There has been little investigation undertaken regarding variation among members of the same species from different geographical locales. In order to investigate the potential variation of these structures, or morphological landmarks, I decided to compare the sizes of identifying features on third instar *Phormia regina* (Meigen) originating from two different locations (Indiana and California). The goal of these measurements was to confirm whether or not there was significant variation in morphology between these two groups. I hypothesized that due to genetic history and other variables, significant variation in morphological landmarks is possible.

Methods

Specimen acquisition: During the Summer of 2017, adult *Phormia regina* were collected on the property of the Entomology Field Operations Building (EFOB) in West Lafayette, IN. These adults were used to create a *P. regina* colony, and when they reproduced, eggs were collected and reared in a separate enclosure. These larvae were allowed to develop until the third instar, and 100 specimens were sacrificed, boiled in water, and preserved in >80% ethanol. Non-local *P. regina* were harvested from the Kimsey Lab Colony, and sent from the University of California in Davis, CA. For both locations, n = 100.

These specimens were later cleared following the protocol of Nigoghosian *et al*, using a 15% KOH solution. Cleared specimens were then stained with lignin pink.

Data acquisition: A set of morphological landmarks located on the posterior of the 12th segment was selected, photographed, and then measured on each specimen using ImageJ. The landmarks chosen were (see Fig. 1) the distance between the upper posterior papillae (P1-P2, P2-P3, P1-P3) on each side (the viewer's left and right sides, respectively), the distance between the anal lobes (AL), and the distance between the spiracles (S) at the widest point. The differences between measurements of the Indiana specimens and the California specimens were then analyzed for statistically significant variation.

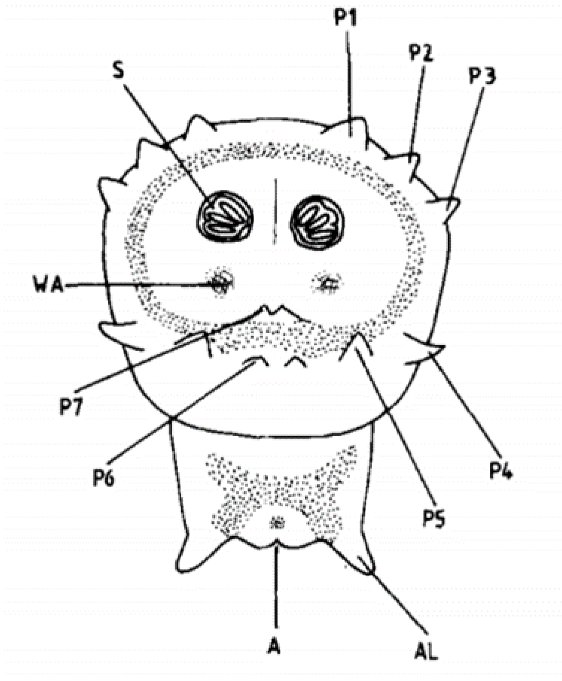


Figure 1. Posterior view of the morphology of 12th segment of third instar larva (A, anus; AL, anal lobe; P1-P7, posterior papillae; S, posterior spiracle; WA, wrinkled area). Image from Erzinçlioğlu, Y.Z. 1985. *Immature stages of British Calliphora and Cynomya, with a re-evaluation of the taxonomic characters of larval Calliphoridae (Diptera).*

Statistical analysis: The data from both sample sets was analyzed using Statistica Academic, as well as the T-Test function in Excel.

Results

Table 1. Measurements (in mm) for each Indiana specimen.

	P1-P2 (L)	P2-P3 (L)	P1-P3 (L)	P1-P2 (R)	P2-P3 (R)	P1-P3 (R)	AL	S
IN1	0.186	0.116	0.335	0.198	0.16	0.471	1.01	0.263
IN2	0.165	0.128	0.299	0.246	0.108	0.379	0.885	0.27
IN3	0.231	0.179	0.468	0.166	0.159	0.333	0.938	0.245
IN4	0.321	0.333	0.659	0.27	0.182	0.485	0.903	0.258
IN5	0.193	0.134	0.365	0.145	0.154	0.342	1.016	0.217
IN6	0.231	0.183	0.444	0.219	0.169	0.502	0.912	0.264
IN7	0.077	0.125	0.236	0.086	0.108	0.231	0.485	0.204
IN8	0.194	0.2023	0.495	0.204	0.191	0.394	1.01	0.311
IN9	0.159	0.117	0.331	0.17	0.13	0.314	0.692	0.196
IN10	0.285	0.131	0.441	0.234	0.194	0.537	1.064	0.284
IN11	0.224	0.151	0.313	0.182	0.166	0.424	1.022	0.288
IN12	0.214	0.182	0.466	0.423	0.166	0.547	1.131	0.283
IN13	0.254	0.141	0.422	0.157	0.126	0.339	0.86	0.248
IN14	0.198	0.16	0.403	0.225	0.086	0.234	1.007	0.232
IN15	0.298	0.182	0.485	0.244	0.158	0.494	1.103	0.248
IN16	0.243	0.174	0.46	0.201	0.185	0.478	1.029	0.249

IN17	0.209	0.078	0.366	0.257	0.121	0.399	0.884	0.259
IN18	0.237	0.145	0.464	0.251	0.155	0.477	1.138	0.285
IN19	0.189	0.146	0.407	0.239	0.143	0.448	1.069	0.248
IN20	0.231	0.138	0.388	0.252	0.161	0.52	0.975	0.242
IN21	0.234	0.154	0.326	0.2	0.14	0.416	0.992	0.281
IN22	0.339	0.212	0.433	0.174	0.128	0.387	0.999	0.257
IN23	0.285	0.176	0.46	0.221	0.152	0.476	1.04	0.323
IN24	0.218	0.155	0.456	0.206	0.186	0.497	1.043	0.228
IN25	0.133	0.14	0.321	0.123	0.119	0.316	0.848	0.254
IN26	0.208	0.142	0.394	0.197	0.172	0.45	0.949	0.273
IN27	0.249	0.155	0.478	0.359	0.18	0.6	0.979	0.277
IN28	0.231	0.164	0.478	0.293	0.177	0.5	1.075	0.349
IN29	0.265	0.154	0.517	0.281	0.222	0.486	0.96	0.306
IN30	0.281	0.193	0.514	0.222	0.193	0.492	1.112	0.292
IN31	0.218	0.16	0.445	0.233	0.157	0.473	1.168	0.338
IN32	0.207	0.243	0.526	0.253	0.156	0.498	0.994	0.221
IN33	0.245	0.135	0.41	0.192	0.124	0.404	0.953	0.241
IN34	0.179	0.181	0.443	0.174	0.201	0.447	0.881	0.172
IN35	0.262	0.126	0.425	0.23	0.109	0.361	0.824	0.245
IN36	0.332	0.14	0.459	0.23	0.166	0.42	0.864	0.27
IN37	0.277	0.124	0.434	0.183	0.122	0.402	0.819	0.288
IN38	0.178	0.172	0.423	0.168	0.169	0.379	0.9	0.243
IN39	0.22	0.169	0.427	0.145	0.087	0.314	0.921	0.239
IN40	0.242	0.143	0.355	0.212	0.181	0.363	0.879	0.286
IN41	0.179	0.144	0.366	0.158	0.144	0.392	0.904	0.258
IN42	0.179	0.133	0.387	0.231	0.187	0.484	1.063	0.252
IN43	0.181	0.158	0.418	0.182	0.097	0.335	1.005	0.199
IN44	0.269	0.135	0.425	0.159	0.138	0.386	0.929	0.234
IN45	0.166	0.127	0.345	0.158	0.177	0.382	1.458	0.246
IN46	0.2	0.157	0.374	0.207	0.17	0.387	0.966	0.259
IN47	0.266	0.125	0.368	0.268	0.17	0.429	0.913	0.249
IN48	0.178	0.16	0.395	0.188	0.136	0.352	0.872	0.27
IN49	0.301	0.213	0.413	0.219	0.143	0.435	0.936	0.291
IN50	0.117	0.16	0.327	0.123	0.155	0.389	0.678	0.209
IN51	0.162	0.189	0.336	0.137	0.131	0.344	0.582	0.202
IN52	0.223	0.179	0.462	0.195	0.153	0.408	0.958	0.213
IN53	0.194	0.177	0.409	0.194	0.14	0.367	0.994	0.197
IN54	0.275	0.145	0.463	0.125	0.148	0.392	0.881	0.221
IN55	0.247	0.102	0.395	0.2	0.186	0.429	0.849	0.212
IN56	0.258	0.178	0.491	0.268	0.147	0.465	1.059	0.237
IN57	0.241	0.22	0.443	0.244	0.112	0.441	0.97	0.228
IN58	0.185	0.093	0.332	0.201	0.091	0.364	0.757	0.261
IN59	0.237	0.157	0.433	0.198	0.107	0.395	1.104	0.271

IN60	0.19	0.174	0.384	0.176	0.13	0.363	1.302	0.221
IN61	0.159	0.126	0.346	0.123	0.184	0.428	1.092	0.281
IN62	0.223	0.154	0.41	0.15	0.154	0.36	1.017	0.262
IN63	0.173	0.138	0.357	0.133	0.145	0.296	0.88	0.244
IN64	0.162	0.139	0.368	0.155	0.182	0.317	1.052	0.281
IN65	0.219	0.113	0.352	0.193	0.144	0.358	0.848	0.249
IN66	0.222	0.108	0.4	0.2	0.139	0.396	0.914	0.21
IN67	0.217	0.129	0.396	0.156	0.125	0.4	0.924	0.265
IN68	0.206	0.125	0.269	0.226	0.141	0.403	1.144	0.271
IN69	0.153	0.132	0.325	0.206	0.158	0.424	0.822	0.231
IN70	0.207	0.133	0.404	0.131	0.083	0.283	0.745	0.274
IN71	0.218	0.174	0.414	0.164	0.164	0.344	0.964	0.224
IN72	0.225	0.168	0.448	0.2	0.17	0.509	0.886	0.23
IN73	0.198	0.176	0.443	0.191	0.199	0.406	0.821	0.296
IN74	0.118	0.107	0.307	0.199	0.118	0.359	0.705	0.213
IN75	0.218	0.146	0.408	0.164	0.114	0.297	0.73	0.196
IN76	0.265	0.141	0.493	0.266	0.158	0.554	0.975	0.238
IN77	0.192	0.139	0.375	0.179	0.193	0.457	0.812	0.239
IN78	0.24	0.103	0.406	0.14	0.199	0.374	0.922	0.237
IN79	0.304	0.175	0.307	0.221	0.171	0.46	1.015	0.304
IN80	0.216	0.106	0.274	0.151	0.275	0.434	0.949	0.29
IN81	0.214	0.154	0.415	0.134	0.12	0.349	0.726	0.223
IN82	0.208	0.172	0.492	0.302	0.126	0.424	0.889	0.247
IN83	0.222	0.128	0.378	0.128	0.121	0.255	1.11	0.261
IN84	0.258	0.23	0.545	0.149	0.123	0.321	0.944	0.278
IN85	0.09	0.098	0.233	0.185	0.123	0.374	0.984	0.201
IN86	0.214	0.158	0.367	0.19	0.137	0.424	0.905	0.223
IN87	0.204	0.158	0.374	0.229	0.137	0.423	0.88	0.225
IN88	0.227	0.154	0.417	0.143	0.171	0.346	0.894	0.276
IN89	0.173	0.148	0.371	0.127	0.094	0.274	0.926	0.241
IN90	0.117	0.128	0.261	0.203	0.117	0.382	0.725	0.225
IN91	0.208	0.158	0.436	0.146	0.157	0.41	0.98	0.257
IN92	0.228	0.113	0.359	0.173	0.119	0.329	0.747	0.209
IN93	0.265	0.155	0.368	0.262	0.154	0.451	0.848	0.238
IN94	0.176	0.133	0.379	0.248	0.104	0.33	0.735	0.23
IN95	0.2	0.11	0.403	0.206	0.155	0.375	0.925	0.254
IN96	0.195	0.163	0.423	0.188	0.127	0.37	0.929	0.249
IN97	0.196	0.171	0.507	0.256	0.203	0.468	0.896	0.176
IN98	0.146	0.094	0.307	0.125	0.17	0.348	0.925	0.266
IN99	0.147	0.111	0.329	0.176	0.15	0.412	0.851	0.23
IN100	0.177	0.151	0.366	0.183	0.118	0.303	0.85	0.183

Table 2. Measurements (in mm) for each California specimen.

	P1-P2 (L)	P2-P3 (L)	P1-P3 (L)	P1-P2 (R)	P2-P3 (R)	P1-P3 (R)	AL	S
CA1	0.186	0.15	0.332	0.207	0.113	0.38	1.053	0.289
CA2	0.168	0.101	0.264	0.221	0.197	0.468	0.992	0.27
CA3	0.317	0.122	0.381	0.235	0.158	0.361	0.964	0.147
CA4	0.188	0.165	0.381	0.242	0.214	0.43	0.988	0.23
CA5	0.198	0.249	0.501	0.168	0.224	0.448	0.973	0.225
CA6	0.167	0.092	0.261	0.216	0.162	0.394	0.764	0.199
CA7	0.192	0.172	0.339	0.208	0.162	0.402	0.974	0.223
CA8	0.296	0.181	0.498	0.224	0.098	0.304	1.048	0.214
CA9	0.14	0.185	0.274	0.18	0.163	0.378	0.698	0.169
CA10	0.197	0.153	0.458	0.182	0.163	0.363	1.059	0.206
CA11	0.276	0.182	0.324	0.148	0.173	0.396	0.701	0.227
CA12	0.231	0.137	0.308	0.161	0.121	0.238	0.679	0.222
CA13	0.331	0.186	0.439	0.288	0.148	0.43	0.887	0.236
CA14	0.23	0.271	0.517	0.155	0.188	0.487	0.949	0.23
CA15	0.145	0.196	0.376	0.256	0.159	0.406	0.905	0.263
CA16	0.139	0.122	0.261	0.212	0.155	0.421	0.927	0.211
CA17	0.228	0.124	0.328	0.211	0.156	0.41	0.931	0.302
CA18	0.266	0.188	0.484	0.204	0.179	0.465	0.979	0.295
CA19	0.223	0.317	0.561	0.331	0.146	0.369	1.097	0.247
CA20	0.204	0.175	0.352	0.235	0.134	0.415	0.898	0.267
CA21	0.187	0.125	0.365	0.21	0.14	0.386	0.988	0.251
CA22	0.218	0.252	0.533	0.14	0.203	0.408	1.039	0.175
CA23	0.228	0.148	0.427	0.301	0.181	0.52	1.017	0.263
CA24	0.184	0.131	0.357	0.193	0.147	0.382	0.855	0.238
CA25	0.198	0.187	0.45	0.134	0.159	0.321	0.922	0.231
CA26	0.139	0.207	0.411	0.163	0.15	0.385	0.934	0.188
CA27	0.127	0.154	0.338	0.248	0.201	0.455	1.009	0.309
CA28	0.385	0.443	0.74	0.232	0.182	0.406	1.009	0.331
CA29	0.268	0.198	0.422	0.129	0.209	0.448	0.672	0.204
CA30	0.158	0.173	0.366	0.143	0.176	0.401	0.788	0.251
CA31	0.192	0.205	0.427	0.247	0.141	0.406	1.012	0.222
CA32	0.102	0.088	0.238	0.155	0.111	0.321	0.509	0.202
CA33	0.32	0.192	0.347	0.189	0.158	0.42	0.836	0.222
CA34	0.181	0.156	0.364	0.154	0.173	0.383	1.141	0.279
CA35	0.284	0.146	0.429	0.319	0.141	0.47	1.186	0.239
CA36	0.168	0.168	0.178	0.219	0.178	0.484	1.114	0.3
CA37	0.146	0.11	0.311	0.21	0.202	0.52	0.704	0.154
CA38	0.273	0.122	0.442	0.273	0.167	0.467	0.896	0.364
CA39	0.228	0.12	0.376	0.182	0.123	0.341	0.723	0.168

CA40	0.205	0.125	0.388	0.128	0.13	0.338	0.789	0.208
CA41	0.135	0.172	0.346	0.164	0.162	0.332	0.697	0.222
CA42	0.16	0.178	0.442	0.169	0.156	0.397	0.985	0.231
CA43	0.214	0.146	0.388	0.191	0.133	0.358	0.828	0.189
CA44	0.409	0.157	0.49	0.193	0.108	0.323	1.075	0.252
CA45	0.184	0.209	0.487	0.147	0.15	0.372	0.971	0.235
CA46	0.189	0.237	0.509	0.175	0.143	0.33	0.946	0.248
CA47	0.167	0.138	0.316	0.176	0.136	0.413	1.042	0.2
CA48	0.15	0.116	0.332	0.179	0.159	0.411	0.837	0.136
CA49	0.161	0.123	0.342	0.189	0.084	0.313	0.772	0.182
CA50	0.202	0.154	0.379	0.131	0.232	0.45	1.101	0.233
CA51	0.066	0.129	0.231	0.157	0.123	0.349	0.736	0.317
CA52	0.122	0.061	0.194	0.118	0.115	0.337	0.539	0.148
CA53	0.178	0.301	0.465	0.163	0.121	0.279	1.009	0.202
CA54	0.199	0.174	0.354	0.117	0.122	0.322	0.781	0.247
CA55	0.239	0.181	0.439	0.174	0.148	0.396	1.122	0.239
CA56	0.098	0.174	0.428	0.181	0.099	0.434	0.839	0.244
CA57	0.212	0.157	0.49	0.162	0.165	0.336	1.05	0.194
CA58	0.299	0.177	0.445	0.158	0.131	0.374	0.952	0.26
CA59	0.177	0.143	0.392	0.175	0.264	0.495	0.951	0.207
CA60	0.255	0.228	0.525	0.199	0.087	0.281	1.139	0.198
CA61	0.161	0.146	0.311	0.139	0.127	0.252	0.875	0.204
CA62	0.162	0.224	0.388	0.19	0.187	0.4	0.853	0.219
CA63	0.175	0.14	0.344	0.234	0.127	0.428	0.839	0.236
CA64	0.162	0.162	0.36	0.268	0.153	0.403	0.577	0.194
CA65	0.209	0.084	0.292	0.143	0.094	0.246	0.445	0.117
CA66	0.181	0.123	0.333	0.167	0.154	0.369	0.9	0.156
CA67	0.154	0.183	0.37	0.152	0.136	0.412	0.775	0.351
CA68	0.132	0.148	0.331	0.163	0.113	0.341	0.508	0.299
CA69	0.07	0.09	0.173	0.189	0.122	0.315	0.523	0.205
CA70	0.287	0.244	0.505	0.143	0.116	0.326	0.775	0.221
CA71	0.244	0.216	0.43	0.203	0.177	0.456	1.043	0.302
CA72	0.253	0.134	0.405	0.144	0.144	0.313	0.859	0.258
CA73	0.167	0.132	0.362	0.225	0.185	0.375	0.795	0.186
CA74	0.238	0.17	0.467	0.215	0.137	0.362	0.994	0.246
CA75	0.167	0.177	0.38	0.211	0.161	0.413	0.651	0.178
CA76	0.218	0.22	0.316	0.365	0.229	0.542	0.788	0.246
CA77	0.152	0.205	0.45	0.244	0.191	0.416	0.671	0.262
CA78	0.185	0.149	0.311	0.114	0.107	0.224	0.697	0.227
CA79	0.176	0.164	0.371	0.207	0.175	0.378	0.871	0.154
CA80	0.102	0.143	0.274	0.093	0.168	0.283	0.724	0.19
CA81	0.189	0.18	0.321	0.144	0.228	0.463	0.601	0.219
CA82	0.251	0.203	0.546	0.159	0.175	0.377	1.091	0.205

CA83	0.069	0.076	0.18	0.136	0.128	0.375	0.757	0.155
CA84	0.113	0.153	0.271	0.066	0.148	0.309	0.589	0.218
CA85	0.243	0.146	0.449	0.088	0.137	0.319	0.562	0.218
CA86	0.246	0.217	0.467	0.211	0.143	0.407	0.818	0.176
CA87	0.225	0.254	0.484	0.225	0.101	0.319	0.791	0.272
CA88	0.251	0.23	0.52	0.244	0.166	0.537	0.928	0.371
CA89	0.302	0.19	0.557	0.295	0.263	0.586	1.054	0.447
CA90	0.2	0.176	0.387	0.207	0.243	0.489	0.808	0.195
CA91	0.207	0.171	0.427	0.18	0.183	0.442	0.632	0.23
CA92	0.235	0.136	0.378	0.197	0.109	0.32	0.902	0.296
CA93	0.141	0.17	0.331	0.227	0.099	0.365	0.752	0.332
CA94	0.185	0.125	0.292	0.129	0.093	0.22	0.646	0.158
CA95	0.179	0.141	0.297	0.171	0.142	0.347	1.045	0.283
CA96	0.146	0.093	0.259	0.158	0.124	0.311	0.717	0.196
CA97	0.18	0.215	0.463	0.201	0.137	0.368	0.939	0.286
CA98	0.106	0.061	0.158	0.07	0.1	0.191	0.368	0.2
CA99	0.248	0.171	0.481	0.18	0.214	0.405	0.522	0.326
CA100	0.267	0.118	0.37	0.136	0.105	0.274	0.645	0.219

Table 3. Descriptive statistics for Indiana sample set.

	Mean	Minimum	Maximum	Std.Dev.
P1-P2 (L)	0.213900	0.077000	0.339000	0.048724
P2-P3 (L)	0.151283	0.078000	0.333000	0.035321
P1-P3 (L)	0.401690	0.233000	0.659000	0.069414
P1-P2 (R)	0.197970	0.086000	0.423000	0.052450
P2-P3 (R)	0.149670	0.083000	0.275000	0.032589
P1-P3 (R)	0.401950	0.231000	0.600000	0.071369
AL	0.933990	0.485000	1.458000	0.137646
S	0.249340	0.172000	0.349000	0.033630

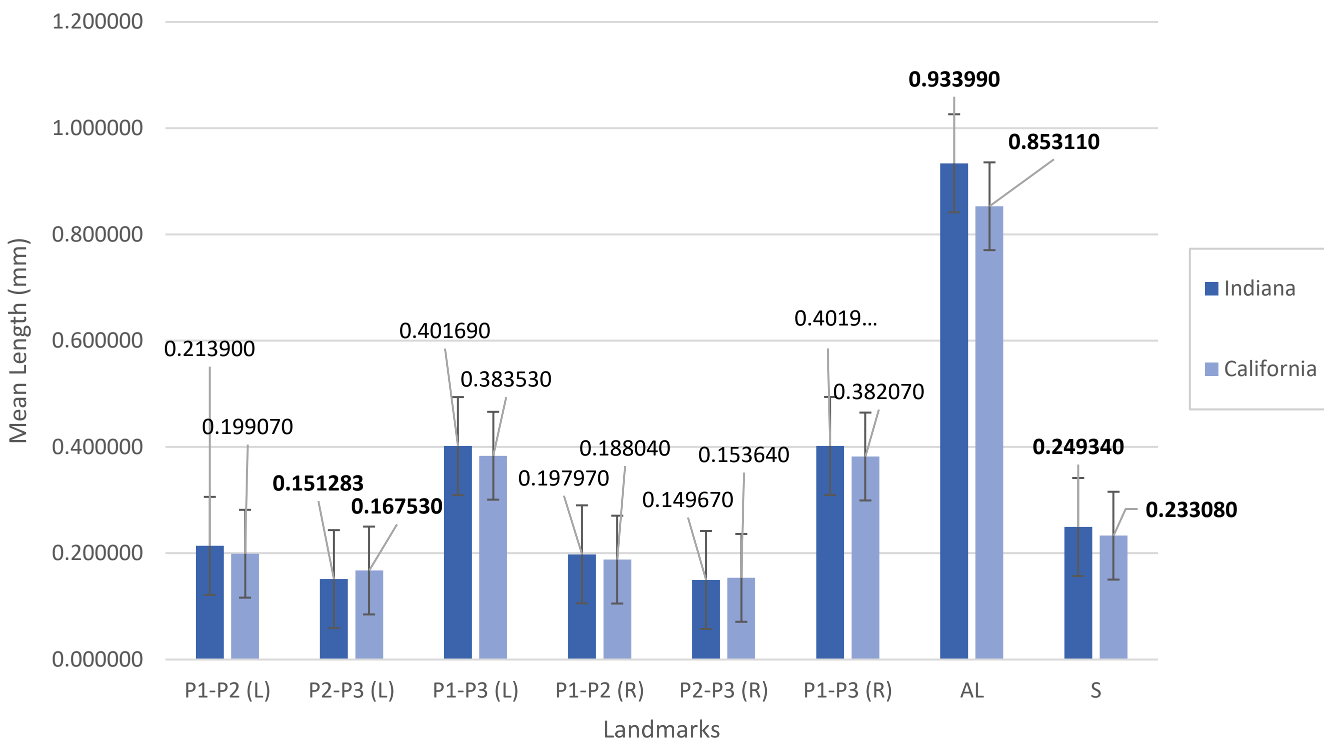
Table 4. Descriptive statistics for California sample set.

	Mean	Minimum	Maximum	Std.Dev.
P1-P2 (L)	0.199070	0.066000	0.409000	0.062892
P2-P3 (L)	0.167530	0.061000	0.443000	0.055214
P1-P3 (L)	0.383530	0.158000	0.740000	0.097506
P1-P2 (R)	0.188040	0.066000	0.365000	0.053064
P2-P3 (R)	0.153640	0.084000	0.264000	0.038576
P1-P3 (R)	0.382070	0.191000	0.586000	0.073124
AL	0.853110	0.368000	1.186000	0.178191
S	0.233080	0.117000	0.447000	0.055083

Table 5. T-Test results. Bolded values indicate statistically significant variation ($p < 0.05$).

	P1-P2 (L)	P2-P3 (L)	P1-P3 (L)	P1-P2 (R)	P2-P3 (R)	P1-P3 (R)	AL	S
p-value	0.06388536	**0.01416917**	0.13096885	0.18475226	0.432743566	0.05312083	**0.00041977**	**0.0127111**

Figure 2. Graphical comparison of the mean values for each landmark in both sample sets. Bolded values indicate statistically significant variation ($p < 0.05$).



Discussion

The results of the T-Test indicate that P2-P3 (L), AL, and S show significant variation between the two sample sets, as they have p-values less than 0.05. Contrary to this, the rest of the landmarks do not show the same level of significance, scoring p-values greater than 0.05.

I am skeptical of the significance of the P2-P3 (L) result, as one would expect the opposite (R) side to be significant as well. This significance could be attributed to human error in measurement, rather than actual variation. The significance of AL and S, however, appears more valid, as these structures are centralized on the segment rather than being peripheral structures.

This information alone is not enough to entirely confirm there is significant variation between the morphology of *P. regina* larvae from different geographical locations. It would be interesting to analyze these same sample sets with geometric morphometrics next, as it would result in more complex statistics and intricate shape comparisons between these sets. Populations from other states should be compared with Indiana and California, respectively, to also determine whether either state harbors populations with particularly divergent morphology. Another viable comparison would be between *P. regina* and other local species that would be present at postmortem sites, such as other Calliphoridae like *Lucilia sericata*.

Acknowledgements

Many thanks to Dr. Trevor Stamper for acting as my research mentor and guiding me through this process. I would also like to thank Gregory Nigoghosian for training me in sample preparation and photography, as well as supplying the clearing SOP used.

References

Erzinçlioğlu, Y.Z. 1985. *Immature stages of British Calliphora and Cynomya, with a re-evaluation of the taxonomic characters of larval Calliphoridae (Diptera).* Journal of Natural History; 19(1), 69-96.

Nuñez, J. & Liria, J. 2016*. Cephalopharyngeal geometric morphometrics in three blowfly species (Diptera: Calliphoridae).* Journal of Entomology and Zoology Studies 2016; 4(1):338-341.